Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US2006/032264

International filing date: 18 August 2006 (18.08.2006)

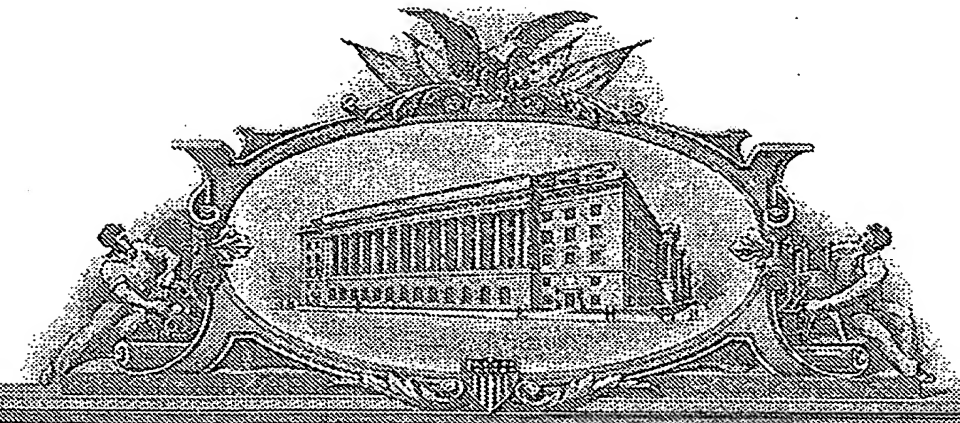
Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/709,861
Filing date: 19 August 2005 (19.08.2005)

Date of receipt at the International Bureau: 10 October 2006 (10.10.2006)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse



1525819

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

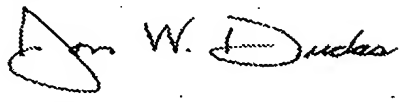
October 03, 2006

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/709,861*
FILING DATE: *August 19, 2005*
RELATED PCT APPLICATION NUMBER: *PCT/US06/32264*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US60/709,861*

Certified by



Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (07-05)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 629341152 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Elliott P. Kristie E.	DAWSON WOMBLE	Murfreesboro, TN Franklin, TN

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max):

METHOD AND SUBSTANCES FOR THE ISOLATION, AMPLIFICATION AND DETECTION OF miRNAs

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ The address corresponding to Customer Number: 23676

OR

☐ Firm or Individual Name David A. Farah, M.D.

Address SHELDON & MAK PC
225 South Lake Avenue, 9th Floor

City Pasadena	State California	Zip 91101
Country United States of America	Telephone (626) 796-4000	Email

ENCLOSED APPLICATION PARTS *(check all that apply)*

☐ Application Data Sheet. See 37 CFR 1.76
☑ Specification *Number of Pages* 39
☑ Drawing(s) *Number of Sheets* 10
☐ CD(s), Number of CDs _____
☒ Other (specify) Return Receipt Postcard

Fees Due: Filing Fee of $200 ($100 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF FILING FEES AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable).
☐ Payment by credit card. Form PTO-2038 is attached
☑ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 19-2090. A duplicative copy of this form is enclosed for fee processing.

$100.00
TOTAL FEE AMOUNT ($)

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
☑ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

SIGNATURE D d Farah
Date August 19, 2005

TYPED or PRINTED NAME David A. Farah, M.D.
REGISTRATION NO. 38,134 *(if appropriate)*

TELEPHONE (626) 796-4000
Docket Number: 16306

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)			Docket No. 16304
Serial No. To be assigned	Filing Date August 19, 2005	Examiner N/A	Group Art Unit N/A

Applicant/Inventor(s): DAWSON, Elliott P. and WOMBLE, Kristie E.

Title of Invention: METHOD AND SUBSTANCES FOR THE ISOLATION, AMPLIFICATION AND DETECTION OF miRNAs

I hereby certify that the following correspondence:

1. United States Provisional Patent Application (38 pages Disclosure and 1 page Claims)
2. Ten (10) Sheets of Drawings (Figures 1-10)
3. Provisional Application for Patent Cover Sheet (Small Entity) (1 page)
4. Return Receipt Postcard

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on: AUGUST 19, 2005 .

(Date)

Marilyn Paik

(Typed or Printed Name of Person Mailing Paper or Fee)

(Signature of Person Mailing Correspondence)

EV629341152US

("Express Mail" Mailing Label No. EV 629341152 US)

METHOD AND SUBSTANCES FOR THE ISOLATION, AMPLIFICATION AND DETECTION OF miRNAs

BACKGROUND

MicroRNAs (miRNA) are small, frequently 18-24 mer polyribonucleotides derived from longer hairpin noncoding transcripts in eukaryotes. miRNAs have been demonstrated to have a significant role in cellular developmental and differentiation pathways. Consequently, understanding and characterizing the temporal, spatial and cellular expression levels and patterns of expression can contribute to definitive understanding of the role of miRNA in cellular development and differentiation in health and diseases.

Presently, miRNAs are isolated from organisms or cells by (1) obtaining total RNA from such samples (2) fractioning the total RNA into subpopulations by gel electrophoresis or chromatographic fractionation and size selective elution (3) cutting the portion of the gel followed by eluting the 18-24 ribonucleotide RNAs from the gel material or collecting the eluted fraction containing single stranded RNA's in the size range of 18-24 ribonucleotides (4) and subsequent isolation of the RNAs by precipitation and downstream processing of the miRNA population for characterization.

Frequently significant amounts of total RNA from rare specimens, such as tumor tissue or biopsy material from an individual must be utilized to obtain miRNA and the ability to obtain additional material such as mRNA from the same specimen can be compromised. In addition, the downstream processing of miRNAs isolated by present methods usually comprise a ligation of additional short single stranded oligonucleotide (RNA OR DNA) adapters to the respective 5' phosphate group of the miRNAs and a subsequent ligation to the respective 3' hydroxyl group of the miRNAs, usually employing an RNA ligase, typically T4 RNA ligase to accomplish the ligations. The adapter modified miRNAs are then purified from the unligated miRNAs and adapters. The purified adapter modified miRNAs are then amplified by polymerase chain reaction(PCR) using reverse transcription PCR (RT-PCR). Following

amplification, the miRNA copies are subjected to detection, quantitation, cloning and sequencing. Because of the large number of steps and the notorious inefficiencies associated with the repeated purification and RNA ligase steps overall recovery of miRNAs can be time consuming, relatively expensive, exhaustive of nonrenewable source material especially from actual tumors and not fully representative of the population of miRNAs expressed within a sample. This is especially true for miRNAs which may be expressed at low levels which may not be detected at all.

In summary the present methods for isolating and identifying miRNAs suffer from several deficiencies that compromise the recovery and characterization of miRNAs. In particular gel purification and size fractionation substantially reduce the recovery of miRNA as well as single strand to single strand ligations are inherently inefficient. These methods are not specific to miRNA therefore can contain siRNA, tRNA, 5S and 5.8SrRNA and degraded RNA. In addition, because miRNAs have 5' phosphate and 3' hydroxyl groups they can both circularize and concatamerize during ligation substantially compromising subsequent recovery and detection. Further, it is now estimated that there may be as many as 400 to over 800 miRNAs in humans. Therefore there is the need for methods and reagents which enable the isolation, recovery, characterization and quantitation of miRNAs in eukaryotes, especially humans to better understand developmental biology, cell differentiation and the relationship of alterations of the expression of miRNAs in both health and disease.

FIGURES

These and other features, aspects and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying figures where:

Figure 1 through Figure 9 depict various steps of some embodiments of the method according to the present invention; and

Figure 10 shows a sequence trace of the miRNA isolated according to the present invention compared to a reference sequence of human miRNA.

DESCRIPTION

According to one embodiment of the present invention, there are provided a method and substances for the isolation, amplification and detection of miRNAs. In one embodiment, the invention is a capture probe or set or plurality of capture probes composed of polynucleotides or polynucleotide analogs. Each capture probe may be constructed having three regions or segments, each with specific purposes. The three segments comprise of a first segment which can have several functions, a second segment complimentary to either a specific miRNA or to miRNAs of specific size and a third segment which can have several functions. The middle segment is preferably a polynucleotide complimentary to and capable of preferentially hybridizing or capturing miRNAs from a mixture of polynucleotides such as a mixture of messenger RNAs, transfer RNAs, ribosomal RNAs and genomic DNA. The third segment may also have several associated functions. Relative to the miRNA capture segment the first segment is 5' or upstream to the second capture segment while the third segment is located 3'to or downstream from the second capture segment. Further the first and third segments also contain polynucleotide sequences which enable the hybridization of complimentary linker probes which are ligated to the captured miRNAs while these are a member of the probe-miRNA duplexes. The linker sites within the probe significantly improve the ligation of linker segments of polynucleotides or polynucleotide analogs to the 5' and 3' ends of the miRNA members of the duplexes formed between the capture probe and the miRNAs. In addition the capture probes can have a functional group at either their respective 5' or 3' ends which enables their selective immobilization to a solid phase. The miRNA-probe complex is separated from other polynucleotides present in a sample by its capture to a solid phase such as by the interaction of biotin with avidin, streptavidin or their modified forms immobilized to a solid phase. Unbound and interfering substances are removed by washing the solid phase miRNA-probe complexes with suitable buffers. The immobilized probe-miRNA complexes are then hybridized with a pair of linker polynucleotides, the first of which is complimentary to the first segment of the capture probe and a second linker polynucleotide

which is complimentary to the third segment of the capture probe. The first linker has a terminal nucleotide at its 3' position with a free 3' hydroxyl group capable of ligation with the 5' phosphate group present in miRNA species. The second linker has a 5' phosphate group (monophosphate or ligatable phosphate analog) which can be ligated to the 3' hydroxyl group present in miRNAs. The linkers are of sufficient length to be within 1-5 bases of the respective 5' and 3' termini of the miRNAs hybridized to the capture probe. Preferably the linker polynucleotides abut or are immediately adjacent to the respective 5' and 3' ends of the captured miRNAs. After sufficient time for the complex comprising of linker 1, linker 2 and the capture probe-miRNA complexes to form or concurrent with their formation, the mixture is treated with a ligase capable of ligating linker 1 and linker 2 to the respective 5' and 3' ends of the miRNAs, preferably T4 polynucleotide ligase in the presence of suitable buffer and essential cofactors. Following sufficient time for the ligation reactions to proceed essentially to completion, the ligase, unreacted linkers and buffers are removed by washing. The in situ synthesized ligated construct comprising of the linker1-miRNA-linker2 polynucleotide sequences comprising the three segments is eluted from the solid phase complex by application of a small volume of solution which is capable of essentially abolishing or substantially reducing the hybridization between the capture probe and the linkers-miRNA constructs. The released linker-miRNA or miRNA-linker constructs are then separated from the solid phase bound capture probes, for example by transfer of the solution containing the linker-miRNA construct to a new or different container. The released linker-miRNA constructs can then be converted to complimentary DNA copies using reverse transcriptase or any enzyme capable of utilizing a DNA-RNA chimera as a template for replication in the presence of a suitable primer for initiation and/or extension. Once the complimentary strands of the linkers-miRNA constructs have been prepared then they can be greatly amplified, for example by PCR using primers complimentary to the linker segments or portions thereof contained in the complex between the linker-miRNA constructs and their respective cDNAs. The amplicons produced by PCR will be representative of the miRNAs present in the sample examined. Labeling

reagents which can introduce a detectable label into the PCR products can be utilized during PCR and the resulting labeled PCR products corresponding to individual miRNAs in the sample can be characterized, identified and their abundance ascertained for example, by hybridization to a microarray corresponding to the miRNAs present in the species from which the sample was obtained. Further details of the miRNA capture probes and linker-adapter oligonucleotides, amplification, solid phase capture and the like are described in more detail below.

a) Design of the first segment of the Capture Probe or the 3'adapter segment.

The purpose of the 3'adapter segment (A1 in Figure 1) of the miRNA capture probe is to facilitate the juxtaposition of the 5'hydroxylated linker (A2 in Figure 1) to the mature miRNA species hybridized to the miRNA complimentary segment of the capture probe and their consequent ligation with one another. Additionally, the 3'adapter segment of the capture probe can be of between 6-50 nucleotides in length and may be composed of ribonucleotides, deoxynucleotides or a chimera of both as well as being composed of their analogs. In addition the 3' adapter segment of the capture probe can contain a polynucleotide synthesis promotor motif for a polynucleotide polymerase or a sequence complimentary to this promoter motif sequence. Such polynucleotide polymerase promoters can be selected from those for such enzymes as the T7, SP6, or T3 DNA dependent RNA polymerases or the type 2 RNA polymerase of E. coli or from the single stranded DNA dependent N4 RNA polymerase. Additionally the 3' adapter segment of the capture probe may contain one or more specific restriction site motifs, preferably restriction site motifs absent from any restriction site motifs that may be present in the DNA analogs of the population of miRNAs being targeted by the overall set of capture probes. For example, restriction enzymes such as BamH I, Hind III, EcoR I, Not I and the like. Further, the 3' terminus of this segment of the capture probe may contain a solid phase binding group to facilitate the immobilization of the capture probe to a solid phase. Preferably this solid phase binding entity is biotin or an analog of biotin capable of binding with avidin or streptavidin or their functional analogs with high affinity (an affinity

constant of between 10e^12 and 10e^20). However, in some circumstances it may be desirable to covalently attach the capture probe to the solid phase by a linker segment which may be reversible or essentially irreversible in its attachment to the solid phase. Such coupling can be facilitated by for example placement of a terminal 5' primary amino group at the 5' terminus of the capture probe and coupling this to a solid phase surface having free carboxyl groups via carbodiimide chemistry. Preferably such coupling methods will be compatible with preserving the hybridization or binding properties of the oligonucleotides or their analogs comprising the capture probe and not interfere with their function as set forth in this application and is well known to those skilled in the art.

b) Selection of mature miRNA sequences

Selection of mature miRNA sequences and design of complimentary sequences for incorporation in capture probes. There is a central repository provided by the Sanger Center to which newly discovered and known miRNA sequences can be submitted for naming and nomenclature assignment as well as placement of the sequences in a data base for archiving and online retrieval via the world wide web (Griffiths-Jones S., "The miRNA Registry", *Nucleic Acids Research*, 32, D109-D111, 2004). The URL for the miRNA Registry web site is: http://miRNA.sanger.ac.uk/sequences/. Generally, the sequences of miRNAs available from this site include species, source, corresponding genomic sequences and genomic location (usually chromosomal coordinates) as well as full length transcription products and sequences for the mature fully processed miRNA sequences.

c) Design of the miRNA complimentary segment of the miRNA Capture Probes, segment two of the Capture Probes.

The miRNA binding segment of the capture probe is the second structural segment of the three contiguous segments composing the capture probe. In order to design capture probes the mature processed forms of the desired miRNA or set of miRNAs is retrieved or otherwise identified. The miRNA registry contains duplicate entries for several of the miRNAs in the set, consequently it is desirable to remove these duplicated entries so that the completed data

set contains single entries for each miRNA. The reverse compliment of the set of mature miRNA sequences is then determined. This set of reverse complimentary miRNA sequences is then preferably converted to their corresponding deoxynucleotide sequences. An example of this for a set of mature human miRNAs is depicted in Table 1.

Table 1

The reverse complimentary RNA sequences and their corresponding DNA sequences directed to a set of human miRNAs.

NAME	REVIEW COMPL miRNA SEQUENCE '5-3'	REV COMPL DNA SEQUENCE '5-3'
>hsa-let-7a	AACUAUACAACCUACUACCUCA	AACTATACAACCTACTACCTCA
>hsa-let-7b	AACCACACAACCUACUACCUCA	AACCACACAACCTACTACCTCA
>hsa-let-7c	AACCAUACAACCUACUACCUCA	AACCATACAACCTACTACCTCA
>hsa-let-7d	ACUAUGCAACCUACUACCUCU	ACTATGCAACCTACTACCTCT
>hsa-let-7e	ACUAUACAACCUCCUACCUCA	ACTATACAACCTCCTACCTCA
>hsa-let-7f	AACUAUACAAUCUACUACCUCA	AACTATACAATCTACTACCTCA
>hsa-let-7g	ACUGUACAAACUACUACCUCA	ACTGTACAAACTACTACCTCA
>hsa-let-7i	ACAGCACAAACUACUACCUCA	ACAGCACAAACTACTACCTCA
>hsa-miR-100	CACAAGUUCGGAUCUACGGGUU	CACAAGTTCGGATCTACGGGTT
>hsa-miR-101	CUUCAGUUAUCACAGUACUGUA	CTTCAGTTATCACAGTACTGTA
>hsa-miR-103	UCAUAGCCCUGUACAAUGCUGCU	TCATAGCCCTGTACAATGCTGCT
>hsa-miR-103	UCAUAGCCCUGUACAAUGCUGCU	TCATAGCCCTGTACAATGCTGCT
>hsa-miR-105	ACAGGAGUCUGAGCAUUUGA	ACAGGAGTCTGAGCATTTGA
>hsa-miR-106a	GCUACCUGCACUGUAAGCACUUUU	GCTACCTGCACTGTAAGCACTTTT
>hsa-miR-106b	AUCUGCACUGUCAGCACUUUA	ATCTGCACTGTCAGCACTTTA
>hsa-miR-107	UGAUAGCCCUGUACAAUGCUGCU	TGATAGCCCTGTACAATGCTGCT
>hsa-miR-10a	CACAAAUUCGGAUCUACAGGGUA	CACAAATTCGGATCTACAGGGTA
>hsa-miR-10b	ACAAAUUCGGUUCUACAGGGUA	ACAAATTCGGTTCTACAGGGTA
>hsa-miR-1	UACAUACUUCUUUACAUUCCA	TACATACTTCTTTACATTCCA
>hsa-miR-122a	ACAAACACCAUUGUCACACUCCA	ACAAACACCATTGTCACACTCCA
>hsa-miR-124a	UGGCAUUCACCGCGUGCCUUAA	TGGCATTCACCGCGTGCCTTAA
>hsa-miR-125a	CACAGGUUAAAGGGUCUCAGGGA	CACAGGTTAAAGGGTCTCAGGGA
>hsa-miR-125b	UCACAAGUUAGGGUCUCAGGGA	TCACAAGTTAGGGTCTCAGGGA
>hsa-miR-126*	CGCGUACCAAAAGUAAUAAUG	CGCGTACCAAAAGTAATAATG
>hsa-miR-127	AGCCAAGCUCAGACGGAUCCGA	AGCCAAGCTCAGACGGATCCGA
>hsa-miR-128a	AAAAGAGACCGGUUCACUGUGA	AAAAGAGACCGGTTCACTGTGA
>hsa-miR-128b	GAAAGAGACCGGUUCACUGUGA	GAAAGAGACCGGTTCACTGTGA
>hsa-miR-129	GCAAGCCCAGACCGCAAAAAG	GCAAGCCCAGACCGCAAAAAG
>hsa-miR-130a	AUGCCCUUUUAACAUUGCACUG	ATGCCCTTTTAACATTGCACTG
>hsa-miR-130b	AUGCCCUUUCAUCAUUGCACUG	ATGCCCTTTCATCATTGCACTG
>hsa-miR-132	CGACCAUGGCUGUAGACUGUUA	CGACCATGGCTGTAGACTGTTA
>hsa-miR-133a	ACAGCUGGUUGAAGGGGACCAA	ACAGCTGGTTGAAGGGGACCAA
>hsa-miR-133b	UAGCUGGUUGAAGGGGACCAA	TAGCTGGTTGAAGGGGACCAA
>hsa-miR-134	CCCUCUGGUCAACCAGUCACA	CCCTCTGGTCAACCAGTCACA

The reverse complimentary RNA sequences and their corresponding DNA sequences directed to a set of human miRNAs.

NAME	REVIEW COMPL miRNA SEQUENCE '5-3'	REV COMPL DNA SEQUENCE '5-3'
>hsa-miR-135a	UCACAUAGGAAUAAAAAGCCAUA	TCACATAGGAATAAAAAGCCATA
>hsa-miR-135b	CACAUAGGAAUGAAAAGCCAUA	CACATAGGAATGAAAAGCCATA
>hsa-miR-136	UCCAUCAUCAAAACAAAUGGAGU	TCCATCATCAAAACAAATGGAGT
>hsa-miR-137	CUACGCGUAUUCUUAAGCAAUA	CTACGCGTATTCTTAAGCAATA
>hsa-miR-138	GAUUCACAACACCAGCU	GATTCACAACACCAGCT
>hsa-miR-139	AGACACGUGCACUGUAGA	AGACACGTGCACTGTAGA
>hsa-miR-140	CUACCAUAGGGUAAAACCACU	CTACCATAGGGTAAAACCACT
>hsa-miR-141	CCAUCUUUACCAGACAGUGUUA	CCATCTTTACCAGACAGTGTTA
>hsa-miR-142-5p	GUAGUGCUUUCUACUUUAUG	GTAGTGCTTTCTACTTTATG
>hsa-miR-143	UGAGCUACAGUGCUUCAUCUCA	TGAGCTACAGTGCTTCATCTCA
>hsa-miR-144	CUAGUACAUCAUCUAUACUGUA	CTAGTACATCATCTATACTGTA
>hsa-miR-145	AAGGGAUUCCUGGGAAAACUGGAC	AAGGGATTCCTGGGAAAACTGGAC
>hsa-miR-146	AACCCAUGGAAUUCAGUUCUCA	AACCCATGGAATTCAGTTCTCA
>hsa-miR-147	GCAGAAGCAUUUCCACACAC	GCAGAAGCATTTCCACACAC
>hsa-miR-148a	ACAAAGUUCUGUAGUGCACUGA	ACAAAGTTCTGTAGTGCACTGA
>hsa-miR-148b	ACAAAGUUCUGUGAUGCACUGA	ACAAAGTTCTGTGATGCACTGA
>hsa-miR-149	GGAGUGAAGACACGGAGCCAGA	GGAGTGAAGACACGGAGCCAGA
>hsa-miR-150	CACUGGUACAAGGGUUGGGAGA	CACTGGTACAAGGGTTGGGAGA
>hsa-miR-151	CCUCAAGGAGCUUCAGUCUAGU	CCTCAAGGAGCTTCAGTCTAGT
>hsa-miR-152	CCCAAGUUCUGUCAUGCACUGA	CCCAAGTTCTGTCATGCACTGA
>hsa-miR-153	UCACUUUUGUGACUAUGCAA	TCACTTTTGTGACTATGCAA
>hsa-miR-154	CGAAGGCAACACGGAUAACCUA	CGAAGGCAACACGGATAACCTA
>hsa-miR-155	CCCCUAUCACGAUUAGCAUUAA	CCCCTATCACGATTAGCATTAA
>hsa-miR-15a	CACAAACCAUUAUGUGCUGCUA	CACAAACCATTATGTGCTGCTA
>hsa-miR-15b	UGUAAACCAUGAUGUGCUGCUA	TGTAAACCATGATGTGCTGCTA
>hsa-miR-16	CGCCAAUAUUUACGUGCUGCUA	CGCCAATATTTACGTGCTGCTA
>hsa-miR-17-5p	ACUACCUGCACUGUAAGCACUUUG	ACTACCTGCACTGTAAGCACTTTG
>hsa-miR-18	UAUCUGCACUAGAUGCACCUUA	TATCTGCACTAGATGCACCTTA
>hsa-miR-181a	ACUCACCGACAGCGUUGAAUGUU	ACTCACCGACAGCGTTGAATGTT
>hsa-miR-181b	CCCACCGACAGCAAUGAAUGUU	CCCACCGACAGCAATGAATGTT
>hsa-miR-181c	ACUCACCGACAGGUUGAAUGUU	ACTCACCGACAGGTTGAATGTT
>hsa-miR-182	UGUGAGUUCUACCAUUGCCAAA	TGTGAGTTCTACCATTGCCAAA
>hsa-miR-183	CAGUGAAUUCUACCAGUGCCAUA	CAGTGAATTCTACCAGTGCCATA
>hsa-miR-184	ACCCUUAUCAGUUCUCCGUCCA	ACCCTTATCAGTTCTCCGTCCA
>hsa-miR-185	GAACUGCCUUUCUCUCCA	GAACTGCCTTTCTCTCCA
>hsa-miR-186	AAGCCCAAAAGGAGAAUUCUUUG	AAGCCCAAAAGGAGAATTCTTTG
>hsa-miR-187	CGGCUGCAACACAAGACACGA	CGGCTGCAACACAAGACACGA
>hsa-miR-188	ACCCUCCACCAUGCAAGGGAUG	ACCCTCCACCATGCAAGGGATG
>hsa-miR-190	ACCUAAUAUAUCAAACAUAUCA	ACCTAATATATCAAACATATCA
>hsa-miR-191	AGCUGCUUUUGGGAUUCCGUUG	AGCTGCTTTTGGGATTCCGTTG
>hsa-miR-192	GGCUGUCAAUUCAUAGGUCAG	GGCTGTCAATTCATAGGTCAG
>hsa-miR-193	CUGGGACUUUGUAGGCCAGUU	CTGGGACTTTGTAGGCCAGTT
>hsa-miR-194	UCCACAUGGAGUUGCUGUUACA	TCCACATGGAGTTGCTGTTACA

The reverse complimentary RNA sequences and their corresponding DNA sequences directed to a set of human miRNAs.

NAME	REVIEW COMPL miRNA SEQUENCE '5-3'	REV COMPL DNA SEQUENCE '5-3'
>hsa-miR-195	GCCAAUAUUUCUGUGCUGCUA	GCCAATATTTCTGTGCTGCTA
>hsa-miR-196a	CCAACAACAUGAAACUACCUA	CCAACAACATGAAACTACCTA
>hsa-miR-196b	CCAACAACAGGAAACUACCUA	CCAACAACAGGAAACTACCTA
>hsa-miR-197	GCUGGGUGGAGAAGGUGGUGAA	GCTGGGTGGAGAAGGTGGTGAA
>hsa-miR-198	CCUAUCUCCCCUCUGGACC	CCTATCTCCCCTCTGGACC
>hsa-miR-199a	GAACAGGUAGUCUGAACACUGGG	GAACAGGTAGTCTGAACACTGGG
>hsa-miR-199b	GAACAGAUAGUCUAAACACUGGG	GAACAGATAGTCTAAACACTGGG
>hsa-miR-19a	UCAGUUUUGCAUAGAUUUGCACA	TCAGTTTTGCATAGATTTGCACA
>hsa-miR-19b	UCAGUUUUGCAUGGAUUUGCACA	TCAGTTTTGCATGGATTTGCACA
>hsa-miR-20	CUACCUGCACUAUAAGCACUUUA	CTACCTGCACTATAAGCACTTTA
>hsa-miR-200a	ACAUCGUUACCAGACAGUGUUA	ACATCGTTACCAGACAGTGTTA
>hsa-miR-200b	GUCAUCAUUACCAGGCAGUAUUA	GTCATCATTACCAGGCAGTATTA
>hsa-miR-200c	CCAUCAUUACCCGGCAGUAUUA	CCATCATTACCCGGCAGTATTA
>hsa-miR-203	CUAGUGGUCCUAAACAUUUCAC	CTAGTGGTCCTAAACATTTCAC
>hsa-miR-204	AGGCAUAGGAUGACAAAGGGAA	AGGCATAGGATGACAAAGGGAA
>hsa-miR-205	CAGACUCCGGUGGAAUGAAGGA	CAGACTCCGGTGGAATGAAGGA
>hsa-miR-206	CCACACACUUCCUUACAUUCCA	CCACACACTTCCTTACATTCCA
>hsa-miR-208	ACAAGCUUUUUGCUCGUCUUAU	ACAAGCTTTTTGCTCGTCTTAT
>hsa-miR-21	UCAACAUCAGUCUGAUAAGCUA	TCAACATCAGTCTGATAAGCTA
>hsa-miR-210	UCAGCCGCUGUCACACGCACAG	TCAGCCGCTGTCACACGCACAG
>hsa-miR-211	AGGCGAAGGAUGACAAAGGGAA	AGGCGAAGGATGACAAAGGGAA
>hsa-miR-212	GGCCGUGACUGGAGACUGUUA	GGCCGTGACTGGAGACTGTTA
>hsa-miR-181a	ACUCACCGACAGCGUUGAAUGUU	ACTCACCGACAGCGTTGAATGTT
>hsa-miR-214	CTGCCTGTCTGTGCCTGCTGT	CTGCCTGTCTGTGCCTGCTGT
>hsa-miR-215	GUCUGUCAAUUCAUAGGUCAU	GTCTGTCAATTCATAGGTCAT
>hsa-miR-216	CACAGUUGCCAGCUGAGAUUA	CACAGTTGCCAGCTGAGATTA
>hsa-miR-217	AUCCAAUCAGUUCCUGAUGCAGUA	ATCCAATCAGTTCCTGATGCAGTA
>hsa-miR-218	ACAUGGUUAGAUCAAGCACAA	ACATGGTTAGATCAAGCACAA
>hsa-miR-219	AGAAUUGCGUUUGGACAAUCA	AGAATTGCGTTTGGACAATCA
>hsa-miR-22	ACAGUUCUUCAACUGGCAGCUU	ACAGTTCTTCAACTGGCAGCTT
>hsa-miR-220	AAAGUGUCAGAUACGGUGUGG	AAAGTGTCAGATACGGTGTGG
>hsa-miR-221	GAAACCCAGCAGACAAUGUAGCU	GAAACCCAGCAGACAATGTAGCT
>hsa-miR-222	GAGACCCAGUAGCCAGAUGUAGCU	GAGACCCAGTAGCCAGATGTAGCT
>hsa-miR-223	GGGGUAUUUGACAAACUGACA	GGGGTATTTGACAAACTGACA
>hsa-miR-224	UAAACGGAACCACUAGUGACUUG	TAAACGGAACCACTAGTGACTTG
>hsa-miR-23a	GGAAAUCCCUGGCAAUGUGAU	GGAAATCCCTGGCAATGTGAT
>hsa-miR-23b	GGUAAUCCCUGGCAAUGUGAU	GGTAATCCCTGGCAATGTGAT
>hsa-miR-189	ACUGAUAUCAGCUCAGUAGGCAC	ACTGATATCAGCTCAGTAGGCAC
>hsa-miR-24	CUGUUCCUGCUGAACUGAGCCA	CTGTTCCTGCTGAACTGAGCCA
>hsa-miR-25	UCAGACCGAGACAAGUGCAAUG	TCAGACCGAGACAAGTGCAATG
>hsa-miR-26a	GCCUAUCCUGGAUUACUUGAA	GCCTATCCTGGATTACTTGAA
>hsa-miR-26b	AACCUAUCCUGAAUUACUUGAA	AACCTATCCTGAATTACTTGAA
>hsa-miR-27a	GCGGAACUUAGCCACUGUGAA	GCGGAACTTAGCCACTGTGAA

The reverse complimentary RNA sequences and their corresponding DNA sequences directed to a set of human miRNAs.

NAME	REVIEW COMPL miRNA SEQUENCE '5-3'	REV COMPL DNA SEQUENCE '5-3'
>hsa-miR-27b	GCAGAACUUAGCCACUGUGAA	GCAGAACTTAGCCACTGTGAA
>hsa-miR-28	CUCAAUAGACUGUGAGCUCCUU	CTCAATAGACTGTGAGCTCCTT
>hsa-miR-296	ACAGGAUUGAGGGGGGGCCCU	ACAGGATTGAGGGGGGGCCCT
>hsa-miR-299	AUGUAUGUGGGACGGUAAACCA	ATGTATGTGGGACGGTAAACCA
>hsa-miR-29a	AACCGAUUUCAGAUGGUGCUA	AACCGATTTCAGATGGTGCTA
>hsa-miR-29b	AACACUGAUUUCAAAUGGUGCUA	AACACTGATTTCAAATGGTGCTA
>hsa-miR-29c	ACCGAUUUCAAAUGGUGCUA	ACCGATTTCAAATGGTGCTA
>hsa-miR-301	GCUUUGACAAUACUAUUGCACUG	GCTTTGACAATACTATTGCACTG
>hsa-miR-302a*	AAAGCAAGUACAUCCACGUUUA	AAAGCAAGTACATCCACGTTTA
>hsa-miR-302b*	AGAAAGCACUUCCAUGUUAAAGU	AGAAAGCACTTCCATGTTAAAGT
>hsa-miR-302c*	CAGCAGGUACCCCCAUGUUAAA	CAGCAGGTACCCCCATGTTAAA
>hsa-miR-302d	ACACUCAAACAUGGAAGCACUUA	ACACTCAAACATGGAAGCACTTA
>hsa-miR-30a-5p	CUUCCAGUCGAGGAUGUUUACA	CTTCCAGTCGAGGATGTTTACA
>hsa-miR-30b	AGCUGAGUGUAGGAUGUUUACA	AGCTGAGTGTAGGATGTTTACA
>hsa-miR-30c	GCUGAGAGUGUAGGAUGUUUACA	GCTGAGAGTGTAGGATGTTTACA
>hsa-miR-30c	GCUGAGAGUGUAGGAUGUUUACA	GCTGAGAGTGTAGGATGTTTACA
>hsa-miR-30d	CUUCCAGUCGGGGAUGUUUACA	CTTCCAGTCGGGGATGTTTACA
>hsa-miR-30e-5p	UCCAGUCAAGGAUGUUUACA	TCCAGTCAAGGATGTTTACA
>hsa-miR-31	CAGCUAUGCCAGCAUCUUGCC	CAGCTATGCCAGCATCTTGCC
>hsa-miR-32	GCAACUUAGUAAUGUGCAAUA	GCAACTTAGTAATGTGCAATA
>hsa-miR-320	UUCGCCCUCUCAACCCAGCUUUU	TTCGCCCTCTCAACCCAGCTTTT
>hsa-miR-323	AGAGGUCGACCGUGUAAUGUGC	AGAGGTCGACCGTGTAATGTGC
>hsa-miR-324-5p	ACACCAAUGCCCUAGGGGAUGCG	ACACCAATGCCCTAGGGGATGCG
>hsa-miR-325	ACACUUACUGGACACCUACUAGG	ACACTTACTGGACACCTACTAGG
>hsa-miR-326	CUGGAGGAAGGGCCCAGAGG	CTGGAGGAAGGGCCCAGAGG
>hsa-miR-328	ACGGAAGGGCAGAGAGGGCCAG	ACGGAAGGGCAGAGAGGGCCAG
>hsa-miR-33	CAAUGCAACUACAAUGCAC	CAATGCAACTACAATGCAC
>hsa-miR-330	UCUCUGCAGGCCGUGUGCUUUGC	TCTCTGCAGGCCGTGTGCTTTGC
>hsa-miR-331	UUCUAGGAUAGGCCCAGGGGC	TTCTAGGATAGGCCCAGGGGC
>hsa-miR-335	ACAUUUUUCGUUAUUGCUCUUGA	ACATTTTTCGTTATTGCTCTTGA
>hsa-miR-337	AAAGGCAUCAUAUAGGAGCUGGA	AAAGGCATCATATAGGAGCTGGA
>hsa-miR-338	UCAACAAAAUCACUGAUGCUGGA	TCAACAAAATCACTGATGCTGGA
>hsa-miR-339	UGAGCUCCUGGAGGACAGGGA	TGAGCTCCTGGAGGACAGGGA
>hsa-miR-340	GGCUAUAAAGUAACUGAGACGGA	GGCTATAAAGTAACTGAGACGGA
>hsa-miR-342	GACGGGUGCGAUUUCUGUGUGAGA	GACGGGTGCGATTTCTGTGTGAGA
>hsa-miR-345	GCCCUGGACUAGGAGUCAGCA	GCCCTGGACTAGGAGTCAGCA
>hsa-miR-346	AGAGGCAGGCAUGCGGGCAGACA	AGAGGCAGGCATGCGGGCAGACA
>hsa-miR-34a	AACAACCAGCUAAGACACUGCCA	AACAACCAGCTAAGACACTGCCA
>hsa-miR-34b	CAAUCAGCUAAUGACACUGCCUA	CAATCAGCTAATGACACTGCCTA
>hsa-miR-34c	GCAAUCAGCUAACUACACUGCCU	GCAATCAGCTAACTACACTGCCT
>hsa-miR-361	GUACCCCUGGAGAUUCUGAUAA	GTACCCCTGGAGATTCTGATAA
>hsa-miR-365	AUAAGGAUUUUUAGGGGCAUUA	ATAAGGATTTTTAGGGGCATTA
>hsa-miR-367	UCACCAUUGCUAAAGUGCAAUU	TCACCATTGCTAAAGTGCAATT

The reverse complimentary RNA sequences and their corresponding DNA sequences directed to a set of human miRNAs.

NAME	REVIEW COMPL miRNA SEQUENCE '5-3'	REV COMPL DNA SEQUENCE '5-3'
>hsa-miR-368	AAACGUGGAAUUUCCUCUAUGU	AAACGTGGAATTTCCTCTATGT
>hsa-miR-369	AAAGAUCAACCAUGUAUUAUU	AAAGATCAACCATGTATTATT
>hsa-miR-370	CCAGGUUCCACCCCAGCAGGC	CCAGGTTCCACCCCAGCAGGC
>hsa-miR-371	ACACUCAAAAGAUGGCGGCAC	ACACTCAAAAGATGGCGGCAC
>hsa-miR-372	ACGCUCAAAUGUCGCAGCACUUU	ACGCTCAAATGTCGCAGCACTTT
>hsa-miR-373*	GGAAAGCGCCCCCAUUUUGAGU	GGAAAGCGCCCCCATTTTGAGT
>hsa-miR-374	CACUUAUCAGGUUGUAUUAUAA	CACTTATCAGGTTGTATTATAA
>hsa-miR-375	UCACGCGAGCCGAACGAACAAA	TCACGCGAGCCGAACGAACAAA
>hsa-miR-376a	ACGUGGAUUUUCCUCUAUGAU	ACGTGGATTTTCCTCTATGAT
>hsa-miR-377	ACAAAAGUUGCCUUUGUGUGAU	ACAAAAGTTGCCTTTGTGTGAT
>hsa-miR-378	ACACAGGACCUGGAGUCAGGAG	ACACAGGACCTGGAGTCAGGAG
>hsa-miR-379	UACGUUCCAUAGUCUACCA	TACGTTCCATAGTCTACCA
>hsa-miR-380-5p	GCGCAUGUUCUAUGGUCAACCA	GCGCATGTTCTATGGTCAACCA
>hsa-miR-381	ACAGAGAGCUUGCCCUUGUAUA	ACAGAGAGCTTGCCCTTGTATA
>hsa-miR-382	CGAAUCCACCACGAACAACUUC	CGAATCCACCACGAACAACTTC
>hsa-miR-383	AGCCACAAUCACCUUCUGAUCU	AGCCACAATCACCTTCTGATCT
>hsa-miR-384	UAUGAACAAUUUCUAGGAAU	TATGAACAATTTCTAGGAAT
>hsa-miR-422a	GGCCUUCUGACCCUAAGUCCAG	GGCCTTCTGACCCTAAGTCCAG
>hsa-miR-423	CUGAGGGGCCUCAGACCGAGCU	CTGAGGGGCCTCAGACCGAGCT
>hsa-miR-424	UUCAAAACAUGAAUUGCUGCUG	TTCAAAACATGAATTGCTGCTG
>hsa-miR-425	GGCGGACACGACAUUCCCGAU	GGCGGACACGACATTCCCGAT
>hsa-miR-429	ACGGUUUUACCAGACAGUAUUA	ACGGTTTTACCAGACAGTATTA
>hsa-miR-448	AUGGGACAUCCUACAUAUGCAA	ATGGGACATCCTACATATGCAA
>hsa-miR-449	ACCAGCUAACAAUACACUGCCA	ACCAGCTAACAATACACTGCCA
>hsa-miR-450	UAUUAGGAACACAUCGCAAAAA	TATTAGGAACACATCGCAAAAA
>hsa-miR-7	CAACAAAAUCACUAGUCUUCCA	CAACAAAATCACTAGTCTTCCA
>hsa-miR-9	UCAUACAGCUAGAUAACCAAAGA	TCATACAGCTAGATAACCAAAGA
>hsa-miR-92	CAGGCCGGGACAAGUGCAAUA	CAGGCCGGGACAAGTGCAATA
>hsa-miR-93	CUACCUGCACGAACAGCACUUU	CTACCTGCACGAACAGCACTTT
>hsa-miR-9	UCAUACAGCUAGAUAACCAAAGA	TCATACAGCTAGATAACCAAAGA
>hsa-miR-95	UGCUCAAUAAAUACCCGUUGAA	TGCTCAATAAATACCCGTTGAA
>hsa-miR-96	GCAAAAAUGUGCUAGUGCCAAA	GCAAAAATGTGCTAGTGCCAAA
>hsa-miR-98	AACAAUACAACUUACUACCUCA	AACAATACAACTTACTACCTCA
>hsa-miR-99a	CACAAGAUCGGAUCUACGGGUU	CACAAGATCGGATCTACGGGTT
>hsa-miR-99b	CGCAAGGUCGGUUCUACGGGUG	CGCAAGGTCGGTTCTACGGGTG
>hsa-miR-126	GCAUUAUUACUCACGGUACGA	GCATTATTACTCACGGTACGA
>hsa-miR-142-3p	UCCAUAAAGUAGGAAACACUACA	TCCATAAAGTAGGAAACACTACA
>hsa-miR-154*	AAUAGGUCAACCGUGUAUGAUU	AATAGGTCAACCGTGTATGATT
>hsa-miR-17-3p	ACAAGUGCCUUCACUGCAGU	ACAAGTGCCTTCACTGCAGT
>hsa-miR-182*	UAGUUGGCAAGUCUAGAACCA	TAGTTGGCAAGTCTAGAACCA
>hsa-miR-199a*	AACCAAUGUGCAGACUACUGUA	AACCAATGTGCAGACTACTGTA
>hsa-miR-213	GGUACAAUCAACGGUCGAUGGU	GGTACAATCAACGGTCGATGGT
>hsa-miR-24	CUGUUCCUGCUGAACUGAGCCA	CTGTTCCTGCTGAACTGAGCCA

The reverse complimentary RNA sequences and their corresponding DNA sequences directed to a set of human miRNAs.

NAME	REVIEW COMPL miRNA SEQUENCE '5-3'	REV COMPL DNA SEQUENCE '5-3'
>hsa-miR-302a	UCACCAAAACAUGGAAGCACUUA	TCACCAAAACATGGAAGCACTTA
>hsa-miR-302b	CUACUAAAACAUGGAAGCACUUA	CTACTAAAACATGGAAGCACTTA
>hsa-miR-302c	CCACUGAAACAUGGAAGCACUUA	CCACTGAAACATGGAAGCACTTA
>hsa-miR-30a-3p	GCUGCAAACAUCCGACUGAAAG	GCTGCAAACATCCGACTGAAAG
>hsa-miR-30e-3p	GCUGUAAACAUCCGACUGAAAG	GCTGTAAACATCCGACTGAAAG
>hsa-miR-324-3p	CCAGCAGCACCUGGGGCAGUGG	CCAGCAGCACCTGGGGCAGTGG
>hsa-miR-373	ACACCCCAAAAUCGAAGCACUUC	ACACCCCAAAATCGAAGCACTTC
>hsa-miR-422b	GGCCUUCUGACUCCAAGUCCAG	GGCCTTCTGACTCCAAGTCCAG
>hsa-miR-380-3p	AAGAUGUGGACCAUAUUACAUA	AAGATGTGGACCATATTACATA
>hsa-miR-9*	ACUUUCGGUUAUCUAGCUUUA	ACTTTCGGTTATCTAGCTTTA

The listing of DNA sequences complimentary to the listing of mature miRNAs to which capture probes are to be directed is then entered into a database file. Preferably each of the sequences is oriented in a 5' to 3' direction during the design process, primarily for convenience as well as being customary in the art. Preferably each of the complimentary sequences, whether the sequence is ultimately composed of ribonucleotides, deoxynucleotides or functional analogs or is of a chimeric composition is capable of hybridization to or binding its respective and corresponding mature miRNA by Watson-Crick base pairing when they are synthesized by standard methods well known in the art for the preparation of oligonucleotides.

d) Design of the third segment of the capture probe or the 5' adapter

The purpose of the 5' adapter segment (B1 in Figure 1) of the miRNA capture probe is to facilitate the juxtaposition of the 5'phosphate group located at the 5' terminus of the linker (B2 in Figure 2) to the 3'' end of the mature miRNA species hybridized to the miRNA complimentary segment of the capture probe and their subsequent ligation with one another. Additionally the 5' adapter segment of the capture probe can be of between 6-50 nucleotides in length and may be composed of ribonucleotides, deoxynucleotides or a chimera of both as well as being composed of their analogs. Additionally, the 3'adapter segment of the capture probe can be of between 6-50 nucleotides in length and may be composed of ribonucleotides, deoxynucleotides or a chimera of both as well as being composed of their analogs. In addition the 5' adapter segment of the

capture probe can contain a polynucleotide synthesis promotor motif for a polynucleotide polymerase or a sequence complimentary to this promoter motif sequence. Such polynucleotide polymerase promoters can be selected from those for such enzymes as the T7, SP6, or T3 DNA dependent RNA polymerases or the type 2 RNA polymerase of E. coli or from the single stranded DNA dependent N4 RNA polymerase. Additionally the 5' adapter segment of the capture probe may contain one or more specific restriction site motifs, preferably restriction site motifs absent from any restriction site motifs that may be present in the DNA analogs of the population of miRNAs being targeted by the overall set of capture probes. For example, restriction enzymes such as BamH I, Hind III, EcoR I, Not I and the like. Further the 5' terminus of this segment of the capture probe may contain a solid phase binding group to facilitate the immobilization of the capture probe to a solid phase. Preferably this solid phase binding entity is biotin or an analog of biotin capable of binding with avidin or streptavidin or their functional analogs with high affinity (an affinity constant of between 10e^12 and 10e^20). However, in some circumstances it may be desirable to covalently attach the capture probe to the solid phase by a linker segment which may be reversible or essentially irreversible in its attachment to the solid phase. Such coupling can be facilitated by for example placement of a terminal 5' primary amino group at the 5' terminus of the capture probe and coupling this to a solid phase surface having free carboxyl groups via carbodiimide chemistry. Preferably such coupling methods will be compatible with preserving the hybridization or binding properties of the oligonucleotides or their analogs comprising the capture probe and not interfere with their function as set forth in this application and is well known to those skilled in the art.

e) Construction and synthesis of the Capture Probes

The capture probe is synthesized as a contiguous single sequence for each miRNA to be isolated from a species or sample. Typically a plurality of sequences will be individually synthesized. The Capture Probe sequences to be synthesized are produced, preferably by concatenating the sequence for the 5' adapter sequence (the third segment) described above with each miRNA complimentary second segment described above followed by concatenation of the

sequence for the 3' adapter sequence (first segment) described above to the concatenated first and second segments. Usually and preferably this series of concatenations is performed in the 5' to 3' orientation for convenience in ordering the list of capture probes from oligonucleotide synthesis vendors such as IDT (Coralville, IA US) or Invitrogen (Carlsbad, CA US) and the like. The resulting synthesis will result in a set or plurality of miRNA capture probes that have identical third segments, variable second segments and identical first segments when the set of capture probes are oriented in their respective 5'-3' orientations.

Table 2, below provides suitable DNA sequence capture probes with 5' biotin for binding to immobilized streptavidin or avidin and identical 5' adapter (third segments) sequence motifs for the SP6 RNA polymerase promoter and variable length/sequence second segments complimentary to specific individual human mature miRNAs, and identical 3' adapter sequence motifs complimentary to the T7 RNA polymerase promoter.

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
>hsa-let-7a	/5BIO/ATTTAGGTGACACTATAGAAACTATACAACCTACTACCTCACCCTATAGT GAGTCGTATTA
>hsa-let-7b	/5BIO/ATTTAGGTGACACTATAGAACCACACAACCTACTACCTCACCCTATAGTG AGTCGTATTA
>hsa-let-7c	/5BIO/ATTTAGGTGACACTATAGAACCATACAACCTACTACCTCACCCTATAGTG AGTCGTATTA
>hsa-let-7d	/5BIO/ATTTAGGTGACACTATAGAACTATGCAACCTACTACCTCTCCCTATAGTG AGTCGTATTA
>hsa-let-7e	/5BIO/ATTTAGGTGACACTATAGAACTATACAACCTCCTACCTCACCCTATAGTG AGTCGTATTA
>hsa-let-7f	/5BIO/ATTTAGGTGACACTATAGAAACTATACAATCTACTACCTCACCCTATAGT GAGTCGTATTA
>hsa-let-7g	/5BIO/ATTTAGGTGACACTATAGAACTGTACAAACTACTACCTCACCCTATAGTG AGTCGTATTA
>hsa-let-7i	/5BIO/ATTTAGGTGACACTATAGAACAGCACAAACTACTACCTCACCCTATAGTG AGTCGTATTA
>hsa-miR-100	/5BIO/ATTTAGGTGACACTATAGACACAAGTTCGGATCTACGGGTTCCCTATAGT GAGTCGTATTA
>hsa-miR-101	/5BIO/ATTTAGGTGACACTATAGACTTCAGTTATCACAGTACTGTACCCTATAGTG AGTCGTATTA
>hsa-miR-103	/5BIO/ATTTAGGTGACACTATAGATCATAGCCCTGTACAATGCTGCTCCCTATAGT GAGTCGTATTA
>hsa-miR-103	/5BIO/ATTTAGGTGACACTATAGATCATAGCCCTGTACAATGCTGCTCCCTATAGT GAGTCGTATTA
>hsa-miR-105	/5BIO/ATTTAGGTGACACTATAGAACAGGAGTCTGAGCATTTGACCCTATAGTGA

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
	GTCGTATTA
>hsa-miR-106a	/5BIO/ATTTAGGTGACACTATAGAGCTACCTGCACTGTAAGCACTTTTCCCTATAG TGAGTCGTATTA
>hsa-miR-106b	/5BIO/ATTTAGGTGACACTATAGAATCTGCACTGTCAGCACTTTACCCTATAGTG AGTCGTATTA
>hsa-miR-107	/5BIO/ATTTAGGTGACACTATAGATGATAGCCCTGTACAATGCTGCTCCCTATAG TGAGTCGTATTA
>hsa-miR-10a	/5BIO/ATTTAGGTGACACTATAGACACAAATTCGGATCTACAGGGTACCCTATAG TGAGTCGTATTA
>hsa-miR-10b	/5BIO/ATTTAGGTGACACTATAGAACAAATTCGGTTCTACAGGGTACCCTATAGT GAGTCGTATTA
>hsa-miR-1	/5BIO/ATTTAGGTGACACTATAGATACATACTTCTTTACATTCCACCCTATAGTGA GTCGTATTA
>hsa-miR-122a	/5BIO/ATTTAGGTGACACTATAGAACAAACACCATTGTCACACTCCACCCTATAG TGAGTCGTATTA
>hsa-miR-124a	/5BIO/ATTTAGGTGACACTATAGATGGCATTCACCGCGTGCCTTAACCCTATAGT GAGTCGTATTA
>hsa-miR-125a	/5BIO/ATTTAGGTGACACTATAGACACAGGTTAAAGGGTCTCAGGGACCCTATAG TGAGTCGTATTA
>hsa-miR-125b	/5BIO/ATTTAGGTGACACTATAGATCACAAGTTAGGGTCTCAGGGACCCTATAGT GAGTCGTATTA
>hsa-miR-126*	/5BIO/ATTTAGGTGACACTATAGACGCGTACCAAAAGTAATAATGCCCTATAGTG AGTCGTATTA
>hsa-miR-127	/5BIO/ATTTAGGTGACACTATAGAAGCCAAGCTCAGACGGATCCGACCCTATAGT GAGTCGTATTA
>hsa-miR-128a	/5BIO/ATTTAGGTGACACTATAGAAAAAGAGACCGGTTCACTGTGACCCTATAGT GAGTCGTATTA
>hsa-miR-128b	/5BIO/ATTTAGGTGACACTATAGAGAAAGAGACCGGTTCACTGTGACCCTATAGT GAGTCGTATTA
>hsa-miR-129	/5BIO/ATTTAGGTGACACTATAGAGCAAGCCCAGACCGCAAAAAGCCCTATAGTG AGTCGTATTA
>hsa-miR-130a	/5BIO/ATTTAGGTGACACTATAGAATGCCCTTTTAACATTGCACTGCCCTATAGTG AGTCGTATTA
>hsa-miR-130b	/5BIO/ATTTAGGTGACACTATAGAATGCCCTTTCATCATTGCACTGCCCTATAGTG AGTCGTATTA
>hsa-miR-132	/5BIO/ATTTAGGTGACACTATAGACGACCATGGCTGTAGACTGTTACCCTATAGT GAGTCGTATTA
>hsa-miR-133a	/5BIO/ATTTAGGTGACACTATAGAACAGCTGGTTGAAGGGGACCAACCCTATAGT GAGTCGTATTA
>hsa-miR-133b	/5BIO/ATTTAGGTGACACTATAGATAGCTGGTTGAAGGGGACCAACCCTATAGTG AGTCGTATTA
>hsa-miR-134	/5BIO/ATTTAGGTGACACTATAGACCCTCTGGTCAACCAGTCACACCCTATAGTG AGTCGTATTA
>hsa-miR-135a	/5BIO/ATTTAGGTGACACTATAGATCACATAGGAATAAAAAGCCATACCCTATAG TGAGTCGTATTA
>hsa-miR-135b	/5BIO/ATTTAGGTGACACTATAGACACATAGGAATGAAAAGCCATACCCTATAGT GAGTCGTATTA

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
>hsa-miR-136	/5BIO/ATTTAGGTGACACTATAGATCCATCATCAAAACAAATGGAGTCCCTATAG TGAGTCGTATTA
>hsa-miR-137	/5BIO/ATTTAGGTGACACTATAGACTACGCGTATTCTTAAGCAATACCCTATAGT GAGTCGTATTA
>hsa-miR-138	/5BIO/ATTTAGGTGACACTATAGAGATTCACAACACCAGCTCCCTATAGTGAGTC GTATTA
>hsa-miR-139	/5BIO/ATTTAGGTGACACTATAGAAGACACGTGCACTGTAGACCCTATAGTGAGT CGTATTA
>hsa-miR-140	/5BIO/ATTTAGGTGACACTATAGACTACCATAGGGTAAAACCACTCCCTATAGTG AGTCGTATTA
>hsa-miR-141	/5BIO/ATTTAGGTGACACTATAGACCATCTTTACCAGACAGTGTTACCCTATAGT GAGTCGTATTA
>hsa-miR-142-5p	/5BIO/ATTTAGGTGACACTATAGAGTAGTGCTTTCTACTTTATGCCCTATAGTGAG TCGTATTA
>hsa-miR-143	/5BIO/ATTTAGGTGACACTATAGATGAGCTACAGTGCTTCATCTCACCCTATAGT GAGTCGTATTA
>hsa-miR-144	/5BIO/ATTTAGGTGACACTATAGACTAGTACATCATCTATACTGTACCCTATAGTG AGTCGTATTA
>hsa-miR-145	/5BIO/ATTTAGGTGACACTATAGAAAGGGATTCCTGGGAAAACTGGACCCCTATA GTGAGTCGTATTA
>hsa-miR-146	/5BIO/ATTTAGGTGACACTATAGAAACCCATGGAATTCAGTTCTCACCCTATAGT GAGTCGTATTA
>hsa-miR-147	/5BIO/ATTTAGGTGACACTATAGAGCAGAAGCATTTCCACACACCCCTATAGTGA GTCGTATTA
>hsa-miR-148a	/5BIO/ATTTAGGTGACACTATAGAACAAAGTTCTGTAGTGCACTGACCCTATAGT GAGTCGTATTA
>hsa-miR-148b	/5BIO/ATTTAGGTGACACTATAGAACAAAGTTCTGTGATGCACTGACCCTATAGT GAGTCGTATTA
>hsa-miR-149	/5BIO/ATTTAGGTGACACTATAGAGGAGTGAAGACACGGAGCCAGACCCTATAGT GAGTCGTATTA
>hsa-miR-150	/5BIO/ATTTAGGTGACACTATAGACACTGGTACAAGGGTTGGGAGACCCTATAGT GAGTCGTATTA
>hsa-miR-151	/5BIO/ATTTAGGTGACACTATAGACCTCAAGGAGCTTCAGTCTAGTCCCTATAGT GAGTCGTATTA
>hsa-miR-152	/5BIO/ATTTAGGTGACACTATAGACCCAAGTTCTGTCATGCACTGACCCTATAGT GAGTCGTATTA
>hsa-miR-153	/5BIO/ATTTAGGTGACACTATAGATCACTTTTGTGACTATGCAACCCTATAGTGA GTCGTATTA
>hsa-miR-154	/5BIO/ATTTAGGTGACACTATAGACGAAGGCAACACGGATAACCTACCCTATAGT GAGTCGTATTA
>hsa-miR-155	/5BIO/ATTTAGGTGACACTATAGACCCCTATCACGATTAGCATTAACCCTATAGT GAGTCGTATTA
>hsa-miR-15a	/5BIO/ATTTAGGTGACACTATAGACACAAACCATTATGTGCTGCTACCCTATAGT GAGTCGTATTA
>hsa-miR-15b	/5BIO/ATTTAGGTGACACTATAGATGTAAACCATGATGTGCTGCTACCCTATAGT GAGTCGTATTA
>hsa-miR-16	/5BIO/ATTTAGGTGACACTATAGACGCCAATATTTACGTGCTGCTACCCTATAGT

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
	GAGTCGTATTA
>hsa-miR-17-5p	/5BIO/ATTTAGGTGACACTATAGAACTACCTGCACTGTAAGCACTTTGCCCTATA GTGAGTCGTATTA
>hsa-miR-18	/5BIO/ATTTAGGTGACACTATAGATATCTGCACTAGATGCACCTTACCCTATAGT GAGTCGTATTA
>hsa-miR-181a	/5BIO/ATTTAGGTGACACTATAGAACTCACCGACAGCGTTGAATGTTCCCTATAG TGAGTCGTATTA
>hsa-miR-181b	/5BIO/ATTTAGGTGACACTATAGACCCACCGACAGCAATGAATGTTCCCTATAGT GAGTCGTATTA
>hsa-miR-181c	/5BIO/ATTTAGGTGACACTATAGAACTCACCGACAGGTTGAATGTTCCCTATAGT GAGTCGTATTA
>hsa-miR-182	/5BIO/ATTTAGGTGACACTATAGATGTGAGTTCTACCATTGCCAAACCCTATAGT GAGTCGTATTA
>hsa-miR-183	/5BIO/ATTTAGGTGACACTATAGACAGTGAATTCTACCAGTGCCATACCCTATAG TGAGTCGTATTA
>hsa-miR-184	/5BIO/ATTTAGGTGACACTATAGAACCCTTATCAGTTCTCCGTCCACCCTATAGTG AGTCGTATTA
>hsa-miR-185	/5BIO/ATTTAGGTGACACTATAGAGAACTGCCTTTCTCTCCACCCTATAGTGAGTC GTATTA
>hsa-miR-186	/5BIO/ATTTAGGTGACACTATAGAAAGCCCAAAAGGAGAATTCTTTGCCCTATAG TGAGTCGTATTA
>hsa-miR-187	/5BIO/ATTTAGGTGACACTATAGACGGCTGCAACACAAGACACGACCCTATAGTG AGTCGTATTA
>hsa-miR-188	/5BIO/ATTTAGGTGACACTATAGAACCCTCCACCATGCAAGGGATGCCCTATAGT GAGTCGTATTA
>hsa-miR-190	/5BIO/ATTTAGGTGACACTATAGAACCTAATATATCAAACATATCACCCTATAGT GAGTCGTATTA
>hsa-miR-191	/5BIO/ATTTAGGTGACACTATAGAAGCTGCTTTTGGGATTCCGTTGCCCTATAGTG AGTCGTATTA
>hsa-miR-192	/5BIO/ATTTAGGTGACACTATAGAGGCTGTCAATTCATAGGTCAGCCCTATAGTG AGTCGTATTA
>hsa-miR-193	/5BIO/ATTTAGGTGACACTATAGACTGGGACTTTGTAGGCCAGTTCCCTATAGTG AGTCGTATTA
>hsa-miR-194	/5BIO/ATTTAGGTGACACTATAGATCCACATGGAGTTGCTGTTACACCCTATAGT GAGTCGTATTA
>hsa-miR-195	/5BIO/ATTTAGGTGACACTATAGAGCCAATATTTCTGTGCTGCTACCCTATAGTGA GTCGTATTA
>hsa-miR-196a	/5BIO/ATTTAGGTGACACTATAGACCAACAACATGAAACTACCTACCCTATAGTG AGTCGTATTA
>hsa-miR-196b	/5BIO/ATTTAGGTGACACTATAGACCAACAACAGGAAACTACCTACCCTATAGTG AGTCGTATTA
>hsa-miR-197	/5BIO/ATTTAGGTGACACTATAGAGCTGGGTGGAGAAGGTGGTGAACCCTATAGT GAGTCGTATTA
>hsa-miR-198	/5BIO/ATTTAGGTGACACTATAGACCTATCTCCCCTCTGGACCCCCTATAGTGAGT CGTATTA
>hsa-miR-199a	/5BIO/ATTTAGGTGACACTATAGAGAACAGGTAGTCTGAACACTGGGCCCTATAG TGAGTCGTATTA

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
>hsa-miR-199b	/5BIO/ATTTAGGTGACACTATAGAGAACAGATAGTCTAAACACTGGGCCCTATAG TGAGTCGTATTA
>hsa-miR-19a	/5BIO/ATTTAGGTGACACTATAGATCAGTTTTGCATAGATTTGCACACCCTATAGT GAGTCGTATTA
>hsa-miR-19b	/5BIO/ATTTAGGTGACACTATAGATCAGTTTTGCATGGATTTGCACACCCTATAGT GAGTCGTATTA
>hsa-miR-20	/5BIO/ATTTAGGTGACACTATAGACTACCTGCACTATAAGCACTTTACCCTATAGT GAGTCGTATTA
>hsa-miR-200a	/5BIO/ATTTAGGTGACACTATAGAACATCGTTACCAGACAGTGTTACCCTATAGT GAGTCGTATTA
>hsa-miR-200b	/5BIO/ATTTAGGTGACACTATAGAGTCATCATTACCAGGCAGTATTACCCTATAG TGAGTCGTATTA
>hsa-miR-200c	/5BIO/ATTTAGGTGACACTATAGACCATCATTACCCGGCAGTATTACCCTATAGT GAGTCGTATTA
>hsa-miR-203	/5BIO/ATTTAGGTGACACTATAGACTAGTGGTCCTAAACATTTCACCCCTATAGT GAGTCGTATTA
>hsa-miR-204	/5BIO/ATTTAGGTGACACTATAGAAGGCATAGGATGACAAAGGGAACCCTATAGT GAGTCGTATTA
>hsa-miR-205	/5BIO/ATTTAGGTGACACTATAGACAGACTCCGGTGGAATGAAGGACCCTATAGT GAGTCGTATTA
>hsa-miR-206	/5BIO/ATTTAGGTGACACTATAGACCACACACTTCCTTACATTCCACCCTATAGTG AGTCGTATTA
>hsa-miR-208	/5BIO/ATTTAGGTGACACTATAGAACAAGCTTTTTGCTCGTCTTATCCCTATAGTG AGTCGTATTA
>hsa-miR-21	/5BIO/ATTTAGGTGACACTATAGATCAACATCAGTCTGATAAGCTACCCTATAGT GAGTCGTATTA
>hsa-miR-210	/5BIO/ATTTAGGTGACACTATAGATCAGCCGCTGTCACACGCACAGCCCTATAGT GAGTCGTATTA
>hsa-miR-211	/5BIO/ATTTAGGTGACACTATAGAAGGCGAAGGATGACAAAGGGAACCCTATAGT GAGTCGTATTA
>hsa-miR-212	/5BIO/ATTTAGGTGACACTATAGAGGCCGTGACTGGAGACTGTTACCCTATAGTG AGTCGTATTA
>hsa-miR-181a	/5BIO/ATTTAGGTGACACTATAGAACTCACCGACAGCGTTGAATGTTCCCTATAG TGAGTCGTATTA
>hsa-miR-214	/5BIO/ATTTAGGTGACACTATAGACTGCCTGTCTGTGCCTGCTGTCCCTATAGTGA GTCGTATTA
>hsa-miR-215	/5BIO/ATTTAGGTGACACTATAGAGTCTGTCAATTCATAGGTCATCCCTATAGTG AGTCGTATTA
>hsa-miR-216	/5BIO/ATTTAGGTGACACTATAGACACAGTTGCCAGCTGAGATTACCCTATAGTG AGTCGTATTA
>hsa-miR-217	/5BIO/ATTTAGGTGACACTATAGAATCCAATCAGTTCCTGATGCAGTACCCTATA GTGAGTCGTATTA
>hsa-miR-218	/5BIO/ATTTAGGTGACACTATAGAACATGGTTAGATCAAGCACAACCCTATAGTG AGTCGTATTA
>hsa-miR-219	/5BIO/ATTTAGGTGACACTATAGAAGAATTGCGTTTGGACAATCACCCTATAGTG AGTCGTATTA
>hsa-miR-22	/5BIO/ATTTAGGTGACACTATAGAACAGTTCTTCAACTGGCAGCTTCCCTATAGT

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
	GAGTCGTATTA
>hsa-miR-220	/5BIO/ATTTAGGTGACACTATAGAAAAGTGTCAGATACGGTGTGGCCCTATAGTG AGTCGTATTA
>hsa-miR-221	/5BIO/ATTTAGGTGACACTATAGAGAAACCCAGCAGACAATGTAGCTCCCTATAG TGAGTCGTATTA
>hsa-miR-222	/5BIO/ATTTAGGTGACACTATAGAGAGACCCAGTAGCCAGATGTAGCTCCCTATA GTGAGTCGTATTA
>hsa-miR-223	/5BIO/ATTTAGGTGACACTATAGAGGGGTATTTGACAAACTGACACCCTATAGTG AGTCGTATTA
>hsa-miR-224	/5BIO/ATTTAGGTGACACTATAGATAAACGGAACCACTAGTGACTTGCCCTATAG TGAGTCGTATTA
>hsa-miR-23a	/5BIO/ATTTAGGTGACACTATAGAGGAAATCCCTGGCAATGTGATCCCTATAGTG AGTCGTATTA
>hsa-miR-23b	/5BIO/ATTTAGGTGACACTATAGAGGTAATCCCTGGCAATGTGATCCCTATAGTG AGTCGTATTA
>hsa-miR-189	/5BIO/ATTTAGGTGACACTATAGAACTGATATCAGCTCAGTAGGCACCCCTATAG TGAGTCGTATTA
>hsa-miR-24	/5BIO/ATTTAGGTGACACTATAGACTGTTCCTGCTGAACTGAGCCACCCTATAGT GAGTCGTATTA
>hsa-miR-25	/5BIO/ATTTAGGTGACACTATAGATCAGACCGAGACAAGTGCAATGCCCTATAGT GAGTCGTATTA
>hsa-miR-26a	/5BIO/ATTTAGGTGACACTATAGAGCCTATCCTGGATTACTTGAACCCTATAGTG AGTCGTATTA
>hsa-miR-26b	/5BIO/ATTTAGGTGACACTATAGAAACCTATCCTGAATTACTTGAACCCTATAGT GAGTCGTATTA
>hsa-miR-27a	/5BIO/ATTTAGGTGACACTATAGAGCGGAACTTAGCCACTGTGAACCCTATAGTG AGTCGTATTA
>hsa-miR-27b	/5BIO/ATTTAGGTGACACTATAGAGCAGAACTTAGCCACTGTGAACCCTATAGTG AGTCGTATTA
>hsa-miR-28	/5BIO/ATTTAGGTGACACTATAGACTCAATAGACTGTGAGCTCCTTCCCTATAGT GAGTCGTATTA
>hsa-miR-296	/5BIO/ATTTAGGTGACACTATAGAACAGGATTGAGGGGGGGCCCTCCCTATAGTG AGTCGTATTA
>hsa-miR-299	/5BIO/ATTTAGGTGACACTATAGAATGTATGTGGGACGGTAAACCACCCTATAGT GAGTCGTATTA
>hsa-miR-29a	/5BIO/ATTTAGGTGACACTATAGAAACCGATTTCAGATGGTGCTACCCTATAGTG AGTCGTATTA
>hsa-miR-29b	/5BIO/ATTTAGGTGACACTATAGAAACACTGATTTCAAATGGTGCTACCCTATAG TGAGTCGTATTA
>hsa-miR-29c	/5BIO/ATTTAGGTGACACTATAGAACCGATTTCAAATGGTGCTACCCTATAGTGA GTCGTATTA
>hsa-miR-301	/5BIO/ATTTAGGTGACACTATAGAGCTTTGACAATACTATTGCACTGCCCTATAGT GAGTCGTATTA
>hsa-miR-302a*	/5BIO/ATTTAGGTGACACTATAGAAAAGCAAGTACATCCACGTTTACCCTATAGT GAGTCGTATTA
>hsa-miR-302b*	/5BIO/ATTTAGGTGACACTATAGAAGAAAGCACTTCCATGTTAAAGTCCCTATAG TGAGTCGTATTA

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
>hsa-miR-302c*	/5BIO/ATTTAGGTGACACTATAGACAGCAGGTACCCCCATGTTAAACCCTATAGT GAGTCGTATTA
>hsa-miR-302d	/5BIO/ATTTAGGTGACACTATAGAACACTCAAACATGGAAGCACTTACCCTATAG TGAGTCGTATTA
>hsa-miR-30a-5p	/5BIO/ATTTAGGTGACACTATAGACTTCCAGTCGAGGATGTTTACACCCTATAGT GAGTCGTATTA
>hsa-miR-30b	/5BIO/ATTTAGGTGACACTATAGAAGCTGAGTGTAGGATGTTTACACCCTATAGT GAGTCGTATTA
>hsa-miR-30c	/5BIO/ATTTAGGTGACACTATAGAGCTGAGAGTGTAGGATGTTTACACCCTATAG TGAGTCGTATTA
>hsa-miR-30c	/5BIO/ATTTAGGTGACACTATAGAGCTGAGAGTGTAGGATGTTTACACCCTATAG TGAGTCGTATTA
>hsa-miR-30d	/5BIO/ATTTAGGTGACACTATAGACTTCCAGTCGGGGATGTTTACACCCTATAGT GAGTCGTATTA
>hsa-miR-30e-5p	/5BIO/ATTTAGGTGACACTATAGATCCAGTCAAGGATGTTTACACCCTATAGTGA GTCGTATTA
>hsa-miR-31	/5BIO/ATTTAGGTGACACTATAGACAGCTATGCCAGCATCTTGCCCCCTATAGTG AGTCGTATTA
>hsa-miR-32	/5BIO/ATTTAGGTGACACTATAGAGCAACTTAGTAATGTGCAATACCCTATAGTG AGTCGTATTA
>hsa-miR-320	/5BIO/ATTTAGGTGACACTATAGATTCGCCCTCTCAACCCAGCTTTTCCCTATAGT GAGTCGTATTA
>hsa-miR-323	/5BIO/ATTTAGGTGACACTATAGAAGAGGTCGACCGTGTAATGTGCCCCTATAGT GAGTCGTATTA
>hsa-miR-324-5p	/5BIO/ATTTAGGTGACACTATAGAACACCAATGCCCTAGGGGATGCGCCCTATAG TGAGTCGTATTA
>hsa-miR-325	/5BIO/ATTTAGGTGACACTATAGAACACTTACTGGACACCTACTAGGCCCTATAG TGAGTCGTATTA
>hsa-miR-326	/5BIO/ATTTAGGTGACACTATAGACTGGAGGAAGGGCCCAGAGGCCCTATAGTGA GTCGTATTA
>hsa-miR-328	/5BIO/ATTTAGGTGACACTATAGAACGGAAGGGCAGAGAGGGCCAGCCCTATAGT GAGTCGTATTA
>hsa-miR-33	/5BIO/ATTTAGGTGACACTATAGACAATGCAACTACAATGCACCCCTATAGTGAG TCGTATTA
>hsa-miR-330	/5BIO/ATTTAGGTGACACTATAGATCTCTGCAGGCCGTGTGCTTTGCCCCTATAGT GAGTCGTATTA
>hsa-miR-331	/5BIO/ATTTAGGTGACACTATAGATTCTAGGATAGGCCCAGGGGCCCCTATAGTG AGTCGTATTA
>hsa-miR-335	/5BIO/ATTTAGGTGACACTATAGAACATTTTTCGTTATTGCTCTTGACCCTATAGT GAGTCGTATTA
>hsa-miR-337	/5BIO/ATTTAGGTGACACTATAGAAAAGGCATCATATAGGAGCTGGACCCTATAG TGAGTCGTATTA
>hsa-miR-338	/5BIO/ATTTAGGTGACACTATAGATCAACAAAATCACTGATGCTGGACCCTATAG TGAGTCGTATTA
>hsa-miR-339	/5BIO/ATTTAGGTGACACTATAGATGAGCTCCTGGAGGACAGGGACCCTATAGTG AGTCGTATTA
>hsa-miR-340	/5BIO/ATTTAGGTGACACTATAGAGGCTATAAAGTAACTGAGACGGACCCTATAG

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
	TGAGTCGTATTA
>hsa-miR-342	/5BIO/ATTTAGGTGACACTATAGAGACGGGTGCGATTTCTGTGTGAGACCCTATA GTGAGTCGTATTA
>hsa-miR-345	/5BIO/ATTTAGGTGACACTATAGAGCCCTGGACTAGGAGTCAGCACCCTATAGTG AGTCGTATTA
>hsa-miR-346	/5BIO/ATTTAGGTGACACTATAGAAGAGGCAGGCATGCGGGCAGACACCCTATAG TGAGTCGTATTA
>hsa-miR-34a	/5BIO/ATTTAGGTGACACTATAGAAACAACCAGCTAAGACACTGCCACCCTATAG TGAGTCGTATTA
>hsa-miR-34b	/5BIO/ATTTAGGTGACACTATAGACAATCAGCTAATGACACTGCCTACCCTATAG TGAGTCGTATTA
>hsa-miR-34c	/5BIO/ATTTAGGTGACACTATAGAGCAATCAGCTAACTACACTGCCTCCCTATAG TGAGTCGTATTA
>hsa-miR-361	/5BIO/ATTTAGGTGACACTATAGAGTACCCCTGGAGATTCTGATAACCCTATAGT GAGTCGTATTA
>hsa-miR-365	/5BIO/ATTTAGGTGACACTATAGAATAAGGATTTTTAGGGGCATTACCCTATAGT GAGTCGTATTA
>hsa-miR-367	/5BIO/ATTTAGGTGACACTATAGATCACCATTGCTAAAGTGCAATTCCCTATAGT GAGTCGTATTA
>hsa-miR-368	/5BIO/ATTTAGGTGACACTATAGAAAACGTGGAATTTCCTCTATGTCCCTATAGT GAGTCGTATTA
>hsa-miR-369	/5BIO/ATTTAGGTGACACTATAGAAAAGATCAACCATGTATTATTCCCTATAGTG AGTCGTATTA
>hsa-miR-370	/5BIO/ATTTAGGTGACACTATAGACCAGGTTCCACCCCAGCAGGCCCCTATAGTG AGTCGTATTA
>hsa-miR-371	/5BIO/ATTTAGGTGACACTATAGAACACTCAAAAGATGGCGGCACCCCTATAGTG AGTCGTATTA
>hsa-miR-372	/5BIO/ATTTAGGTGACACTATAGAACGCTCAAATGTCGCAGCACTTTCCCTATAG TGAGTCGTATTA
>hsa-miR-373*	/5BIO/ATTTAGGTGACACTATAGAGGAAAGCGCCCCCATTTTGAGTCCCTATAGT GAGTCGTATTA
>hsa-miR-374	/5BIO/ATTTAGGTGACACTATAGACACTTATCAGGTTGTATTATAACCCTATAGTG AGTCGTATTA
>hsa-miR-375	/5BIO/ATTTAGGTGACACTATAGATCACGCGAGCCGAACGAACAAACCCTATAGT GAGTCGTATTA
>hsa-miR-376a	/5BIO/ATTTAGGTGACACTATAGAACGTGGATTTTCCTCTATGATCCCTATAGTGA GTCGTATTA
>hsa-miR-377	/5BIO/ATTTAGGTGACACTATAGAACAAAAGTTGCCTTTGTGTGATCCCTATAGT GAGTCGTATTA
>hsa-miR-378	/5BIO/ATTTAGGTGACACTATAGAACACAGGACCTGGAGTCAGGAGCCCTATAGT GAGTCGTATTA
>hsa-miR-379	/5BIO/ATTTAGGTGACACTATAGATACGTTCCATAGTCTACCACCCTATAGTGAG TCGTATTA
>hsa-miR-380-5p	/5BIO/ATTTAGGTGACACTATAGAGCGCATGTTCTATGGTCAACCACCCTATAGT GAGTCGTATTA
>hsa-miR-381	/5BIO/ATTTAGGTGACACTATAGAACAGAGAGCTTGCCCTTGTATACCCTATAGT GAGTCGTATTA

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
>hsa-miR-382	/5BIO/ATTTAGGTGACACTATAGACGAATCCACCACGAACAACTTCCCCTATAGT GAGTCGTATTA
>hsa-miR-383	/5BIO/ATTTAGGTGACACTATAGAAGCCACAATCACCTTCTGATCTCCCTATAGT GAGTCGTATTA
>hsa-miR-384	/5BIO/ATTTAGGTGACACTATAGATATGAACAATTTCTAGGAATCCCTATAGTGA GTCGTATTA
>hsa-miR-422a	/5BIO/ATTTAGGTGACACTATAGAGGCCTTCTGACCCTAAGTCCAGCCCTATAGT GAGTCGTATTA
>hsa-miR-423	/5BIO/ATTTAGGTGACACTATAGACTGAGGGGCCTCAGACCGAGCTCCCTATAGT GAGTCGTATTA
>hsa-miR-424	/5BIO/ATTTAGGTGACACTATAGATTCAAAACATGAATTGCTGCTGCCCTATAGT GAGTCGTATTA
>hsa-miR-425	/5BIO/ATTTAGGTGACACTATAGAGGCGGACACGACATTCCCGATCCCTATAGTG AGTCGTATTA
>hsa-miR-429	/5BIO/ATTTAGGTGACACTATAGAACGGTTTTACCAGACAGTATTACCCTATAGT GAGTCGTATTA
>hsa-miR-448	/5BIO/ATTTAGGTGACACTATAGAATGGGACATCCTACATATGCAACCCTATAGT GAGTCGTATTA
>hsa-miR-449	/5BIO/ATTTAGGTGACACTATAGAACCAGCTAACAATACACTGCCACCCTATAGT GAGTCGTATTA
>hsa-miR-450	/5BIO/ATTTAGGTGACACTATAGATATTAGGAACACATCGCAAAAACCCTATAGT GAGTCGTATTA
>hsa-miR-7	/5BIO/ATTTAGGTGACACTATAGACAACAAAATCACTAGTCTTCCACCCTATAGT GAGTCGTATTA
>hsa-miR-9	/5BIO/ATTTAGGTGACACTATAGATCATACAGCTAGATAACCAAAGACCCTATAG TGAGTCGTATTA
>hsa-miR-92	/5BIO/ATTTAGGTGACACTATAGACAGGCCGGGACAAGTGCAATACCCTATAGTG AGTCGTATTA
>hsa-miR-93	/5BIO/ATTTAGGTGACACTATAGACTACCTGCACGAACAGCACTTTCCCTATAGT GAGTCGTATTA
>hsa-miR-9	/5BIO/ATTTAGGTGACACTATAGATCATACAGCTAGATAACCAAAGACCCTATAG TGAGTCGTATTA
>hsa-miR-95	/5BIO/ATTTAGGTGACACTATAGATGCTCAATAAATACCCGTTGAACCCTATAGT GAGTCGTATTA
>hsa-miR-96	/5BIO/ATTTAGGTGACACTATAGAGCAAAAATGTGCTAGTGCCAAACCCTATAGT GAGTCGTATTA
>hsa-miR-98	/5BIO/ATTTAGGTGACACTATAGAAACAATACAACTTACTACCTCACCCTATAGT GAGTCGTATTA
>hsa-miR-99a	/5BIO/ATTTAGGTGACACTATAGACACAAGATCGGATCTACGGGTTCCCTATAGT GAGTCGTATTA
>hsa-miR-99b	/5BIO/ATTTAGGTGACACTATAGACGCAAGGTCGGTTCTACGGGTGCCCTATAGT GAGTCGTATTA
>hsa-miR-126	/5BIO/ATTTAGGTGACACTATAGAGCATTATTACTCACGGTACGACCCTATAGTG AGTCGTATTA
>hsa-miR-142-3p	/5BIO/ATTTAGGTGACACTATAGATCCATAAAGTAGGAAACACTACACCCTATAG TGAGTCGTATTA
>hsa-miR-154*	/5BIO/ATTTAGGTGACACTATAGAAATAGGTCAACCGTGTATGATTCCCTATAGT

MiRNA CAPTURE PROBE SEQUENCES

NAME	CAPTURE PROBE DNA SEQUENCES 5' TO 3'
	GAGTCGTATTA
>hsa-miR-17-3p	/5BIO/ATTTAGGTGACACTATAGAACAAGTGCCTTCACTGCAGTCCCTATAGTGA GTCGTATTA
>hsa-miR-182*	/5BIO/ATTTAGGTGACACTATAGATAGTTGGCAAGTCTAGAACCACCCTATAGTG AGTCGTATTA
>hsa-miR-199a*	/5BIO/ATTTAGGTGACACTATAGAAACCAATGTGCAGACTACTGTACCCTATAGT GAGTCGTATTA
>hsa-miR-213	/5BIO/ATTTAGGTGACACTATAGAGGTACAATCAACGGTCGATGGTCCCTATAGT GAGTCGTATTA
>hsa-miR-24	/5BIO/ATTTAGGTGACACTATAGACTGTTCCTGCTGAACTGAGCCACCCTATAGT GAGTCGTATTA
>hsa-miR-302a	/5BIO/ATTTAGGTGACACTATAGATCACCAAAACATGGAAGCACTTACCCTATAG TGAGTCGTATTA
>hsa-miR-302b	/5BIO/ATTTAGGTGACACTATAGACTACTAAAACATGGAAGCACTTACCCTATAG TGAGTCGTATTA
>hsa-miR-302c	/5BIO/ATTTAGGTGACACTATAGACCACTGAAACATGGAAGCACTTACCCTATAG TGAGTCGTATTA
>hsa-miR-30a-3p	/5BIO/ATTTAGGTGACACTATAGAGCTGCAAACATCCGACTGAAAGCCCTATAGT GAGTCGTATTA
>hsa-miR-30e-3p	/5BIO/ATTTAGGTGACACTATAGAGCTGTAAACATCCGACTGAAAGCCCTATAGT GAGTCGTATTA
>hsa-miR-324-3p	/5BIO/ATTTAGGTGACACTATAGACCAGCAGCACCTGGGGCAGTGGCCCTATAGT GAGTCGTATTA
>hsa-miR-373	/5BIO/ATTTAGGTGACACTATAGAACACCCCAAAATCGAAGCACTTCCCCTATAG TGAGTCGTATTA
>hsa-miR-422b	/5BIO/ATTTAGGTGACACTATAGAGGCCTTCTGACTCCAAGTCCAGCCCTATAGT GAGTCGTATTA
>hsa-miR-380-3p	/5BIO/ATTTAGGTGACACTATAGAAAGATGTGGACCATATTACATACCCTATAGT GAGTCGTATTA
>hsa-miR-9*	/5BIO/ATTTAGGTGACACTATAGAACTTTCGGTTATCTAGCTTTACCCTATAGTGA GTCGTATTA

Table 3

Set of Capture Probes Capable of Capturing Both Known and Unknown miRNAs.

GENERAL miRNA CAPTURE PROBES

NAME	SEQUENCE	SIZE
18 MER CAPT PROBE V1	/5BIO/ATTTAGGTGACACTATAGNNNNNNNNNNNNNNNNNCCCTATAGTGAGTCGTATTA	62
19 MER CAPT PROBE V1	/5BIO/ATTTAGGTGACACTATAGNNNNNNNNNNNNNNNNNNNCCCTATAGTGAGTCGTATTA	63
20 MER CAPT PROBE V1	/5BIO/ATTTAGGTGACACTATAGNNNNNNNNNNNNNNNNNNNNCCCTATAGTGAGTCGTATTA	64
21 MER CAPT PROBE V1	/5BIO/ATTTAGGTGACACTATAGNNNNNNNNNNNNNNNNNNN	65

Set of Capture Probes Capable of Capturing Both Known and Unknown miRNAs.

GENERAL miRNA CAPTURE PROBES

NAME	SEQUENCE	SIZE
	NNNCCCTATAGTGAGTCGTATTA	
22 MER CAPT PROBE V1	/5BIO/ATTTAGGTGACACTATAGNNNNNNNNNNNNNNNNNNNNNCCCTATAGTGAGTCGTATTA	66
23 MER CAPT PROBE V1	/5BIO/ATTTAGGTGACACTATAGNNNNNNNNNNNNNNNNNNNNNCCCTATAGTGAGTCGTATTA	67
24 MER CAPT PROBE V1	/5BIO/ATTTAGGTGACACTATAGNNNNNNNNNNNNNNNNNNNNNCCCTATAGTGAGTCGTATTA	68

f) Design and synthesis of the ligatable 5' linker sequence (A2 in Figure 2)

The ligatable linker sequence is composed of a polynucleotide sequence usually deoxyribonucleotides or ribonucleotides or analogs thereof. This segment may be from 6-50 nucleotides in length and is capable of hybridization to a portion or the entirety of adapter segment A1 of the capture probe as illustrated in Figure 1. The portion of the linker which binds with the third adapter portion of the capture probe should be essentially complimentary to this segment of the capture probe especially at its 3' end. However some mismatch in the 5' end of the linker can be accommodated provided that the 3' most 10-15 nucleotides of the linker essentially perfectly hybridize with the corresponding portion of the adapter segment 3 of the capture probe. This linker (ligatable 5' linker sequence) will usually be located 1-5 nucleotides from the 5' phosphorylated end of the miRNA hybridized to the capture probe. More usually it will abut or be immediately adjacent to the 5'phosphorylated end of the miRNA bound or hybridized to the capture probe. Additionally, The 5' linker adapter can be composed of nuclease resistant nucleotides or the phosphate backbone of the linker can be of a phosphothioate nature to render the linker resistant to nuclease degradation. In addition this linker can be prepared so that it may have additional sequences at its 5' end such that it is flush, recessed or overhangs the 3' end of the third segment of the capture probe. If it is designed with an overhang then the overhang portion can serve to generate a primer binding site that permits the distinction of this linker from that of the third segment adapter sequence of the capture probe, for example in downstream amplification reactions. An example of such a linker with an overhang is one

composed of from the 5' end to 3' end comprising sequences of a T3 promotor sequence, a short spacer sequence and a T7 promotor sequencet with the corresponding adapter segment 3 of the capture probe consiting entirely of the compliment of the T7 promotor sequence. Usually the 5' and 3' ends of this linker will be hydroxyl. However the 5' end may be blocked or contain a functional group such as a label such as a fluorescent dye or other detectable or even a binding entity such as biotin. Once designed the sequence can be readily prepared by phosphoramidite chemistry or ordered from a vendor as previously described for the synthesis of the capture probes above.

g) Design and synthesis of the ligatable 3 ' linker sequence (B2 in Figure 2)

The ligatable linker sequence is composed of a polynucleotide sequence usually deoxyribonucleotides or Ribonucleotides or analogs thereof. This segment may be from 6-50 nucleotides in length and is capable of hybridization to a portion or the entirety of adapter segment B1 of the capture probe as illustrated in Figure 1. The portion of the linker which binds with the first adapter portion of the capture probe should be essentially complimentary to this segment of the capture probe especially at its 5' end. However some mismatch in the 3' end of the linker can be accommodated provided that the 5' most 10-15 nucleotides of the linker essentially perfectly hybridize with the corresponding portion of the adapter segment 1 (B1 in Figure 1) of the capture probe. This linker (ligatable 3' linker sequence) will usually be located 1-5 nucleotides from the 3' hydroxyl end of the miRNA hybridized to the capture probe. More usually it will abut or be immediately adjacent to the 3' hydroxyl end of the miRNA bound or hybridized to the capture probe. Additionally, The 3' linker adapter can be composed of nuclease resistant nucleotides or the phosphate backbone of the linker can be of a phosphothioate nature to render the linker resistant to nuclease degradation. In addition this linker can be prepared so that it may have additional sequences at its 5' end such that it is flush, recessed or overhangs the 5' end of the first segment of the capture probe. If it is designed with an overhang then the overhang portion can serve to generate a primer binding site that permits the distinction of this linker from that of the third segment adapter sequence of the capture probe, for example in

downstream amplification reactions. An example of such a linker with an overhang is one composed of from the 5' end to 3' end comprising of sequences of a T3 promotor complimentary sequence, a short spacer sequence and a SP6 promotor complimentary sequence with the corresponding adapter segment 1 of the capture probe consisting entirely of an SP6 promotor sequence. The 5' end of this linker should be phosphorylated or can be a nucleotide with a 5' pyrophosphate bond between it and its adjacent 3' nucleotide of the linker sequence. The 5' phosphate or pyrophosphate nucleotide at this 5' position of the linker enables the ligation of the linker to the 3' hydroxyl group of the hybridized miRNA by the use of for example T4 ligase. Usually the 3' ends of this linker will be hydroxyl. However the 3' end may be blocked or contain a functional group such as a label such as a fluorescent dye or other detectable or even a binding entity such as biotin. Once designed the sequence can be readily prepared by phosphoramidite chemistry or ordered from a vendor as previously described for the synthesis of the capture probes above.

h) Use of the capture probes

Once prepared, the capture probes and ligatable linkers are brought into solution at for example 100pMoles/ul in 1X TE buffer. The specificity of the capture probes for their respective miRNA targets enables their ability to be utilized directly on isolated total RNA without requiring the separation of the population of miRNAs by either gel or chromatographic purification. Total RNA can be obtained from eukaryotic cells, tissues or organisms by means well known in the art. The resuspended miRNA capture probe or plurality of probes is prepared by preparing a solution of the capture probes in 1XTE with each probe a a concentration of between 0.1-10pMoles /ul. The total RNA and the mixture of capture probes are combined or contacted with one another in a hybridization buffer containing RNAse inhibitors such as lithium dodecylsulfate (LiDS) and or 1-100mM aurine tricarboxylic acid usually as its ammonium or sodium salts in a buffer such as .1M MOPS pH 6.5-7.5, 1mM EDTA and 100mM NaCl. Other suitable buffers for hybridization are well known in the art. The capture probes are permitted to incubate with the total RNA for from 1-30 minutes at 25-50C until substantially all of the miRNA has hybridized with the capture

probes. If the capture probes have been immobilized to a solid phase such as beads then the incubation described above is conducted between the total RNA and the solid phase bound capture probes. If the capture probes contain biotin or other affinity labels then the capture probes are bound to a solid phase such as beads by the interaction of the affinity label and its immobilized binding entity, usually to a bead of between 0.01-5 microns in diameter. For example if the capture probes contain biotin at their respective 3' ends then the hybridization reaction mixture is mixed with streptavidin immobilized to 1.0 micron paramagnetic particles. This mixture is incubated for 1-60 minutes at 25-50C and the complexes between miRNAs, capture probes and biotin-streptavidin-beads are separated from the other species in the mixture by the application of a magnetic field of sufficient strength to attract the particles or beads to the surface of the vessel containing the mixture. The supernatant mixture is removed. The beads are washed with buffers suitable to preserve and maintain the hybridization of the miRNAs to their capture probes and remove contaminating substances such as DNA, other RNAs proteins and the like. The beads are then incubated with the two linkers (A2 and B2 in Figure 2) complimentary to segments 1 and 3 of the capture probes (A1 and B1 in Figure 1) to permit their hybridization or binding to the solid phase bound complex between the capture probes and the miRNAs . The beads are then washed in 1X ligation buffer, usually T4 ligase incubation buffer containing ATP available from a variety of manufactures such as Promega, New England Biolabs and the like. This wash is followed by incubation of the newly formed linker-miRNA-capture probe complexes bound to the solid phase with !X T4 ligase buffer containing T4 ligase under conditions of time and temperature well known in the art to accomplish the covalent coupling of the two linkers with miRNAs within the solid phase bound complexes. The mixture is washed to separate reaction components from the solid phase bound complexes. The ligated linker-miRNA species are then eluted from the solid phase by dehybridizing these strands from those of the capture probes bound to the solid phase by application of a low ionic strength solution such as sterile nuclease free water warmed to 80C. The ligated linker-miRNA species are removed from the solid phase by transferring the low ionic strength solution containing them to another vessel. These linker-miRNA species can then be

reverse transcribed or otherwise processed and identified. If the linkers used in producing the ligated linker-miRNA constructs or species are nuclease resistant then any coeluted DNA capture probes can be destroyed by DNAase digestion to eliminate false signals, especially during downstream amplification such as PCR.

i) Reverse Transcription of the probes

Reverse transcription of the recovered ligated linker-miRNA species is accomplished by annealing a primer of 16-25 nucleotides complimentary to the 3' linker ligated to the miRNAs. The primer is usually DNA in nature with the 3' portion of the primer capable of being extended by the action of a polynucleotide polymerase such as a Reverse Transcriptase that can utilize the RNA portion of the ligated linker-miRNA species as a template for extension and chain synthesis. For example, if the 3' linker ligated to the miRNAs is complimentary to the SP6 promotor, then the primer will have the SP6 promotor sequence and will bind to this portion of the linker-miRNA species. In the presence of reverse transcriptase and suitable buffers, cofactors and dNTPs (dA, dG, dC, dT or dU), the primer will be extended producing a faithful copy of each ligated linker-miRNA species. The resulting products will be of the form depicted in Figure 7 comprising usually of a cDNA copy of each linker-miRNA species as a duplex pair of molecules. These duplex molecules can then be utilized in other downstream methods such as PCR.

j) Circularization of the isolated ligated linker-miRNA species

Intrastrand circularization of the eluted linker-miRNA species can be accomplished if the linkers have free 5' and 3' hydroxyl groups by treating these species with T4 polynucleotide kinase to phosphorylate the 5' ends of the molecules. This can be followed by the treatment of the 5'phosphorylated species with CircLigase single strand DNA ligase available from Epicentre, Madison, WI US following the manufactures suggested protocol and reagents for circular ligation. The treatment with T4 polynucleotide kinase will be unnecessary if the 5' linker has a 5'phosphate group and the 3' linker has a free 3' hydroxyl group. Such circularization will render the circular strands impervious to the action of exonucleases. Therefore any coeluted capture probes can be destroyed by the action of single strand specific 3' exonuclease such

exonuclease I from E. coli. Destruction of any co-eluted capture probe will eliminate any false positive signal or detection arising from any traces of the capture probe in the eluted ligated linker-miRNA solution. The circularized ligated linker-miRNA species can then be used to produce RNA runoff sequences using N4 RNA polymerase provided that the N4 RNA polymerase promoter was incorporated into either linker ligated with the miRNAs. N4 RNA polymerase is single stranded DNA dependent RNA polymerase available from Epicentre, Madison, WI. In fact the single stranded is not known to transcribe from a chimeric RNA-DNA molecule such as the ligated linker-miRNA species eluted from the capture probes. Surprisingly buffers, divalent cation and other conditions will be found that enable the ssDNA dependent RNA polymerase to produce RNA transcripts from chimeric RNA-DNA chimeric molecules such as the ligated linker-miRNA species.

k) PCR of cDNA produced from the isolated linker-miRNA species

By selecting appropriate primer pairs for the 5' and 3' ends of the cDNA produced from the ligated linker-miRNAs, PCR can be accomplished. These conditions are well known to those skilled in the art and can be used for example to increase the abundance of the representation of the miRNAs, to incorporate labels for detection, isolation and identification of the miRNAs present in the original sample. Additionally the PCR products can be cloned and sequenced or cloned and cultured as isolated colonies with the subsequent identification of each miRNA present and cells from the isolated colonies grown in perpetuity to provide a library of miRNAs for further study or for the production of RNAi molecules for each miRNA. Such methods are well known in the art.

The use of the materials described in this disclosure is described in the following example.

EXAMPLES

Example 1:

In order to isolate sequence specific miRNAs from other small nucleic acids, capture probes were designed (table 1) to be specifically complimentary to known human miRNAs with the addition of complimentary linker sequences and a biotin attached to the 5' end to act as a solid

phase binding moiety. The miRNA capture probes were obtained from Integrated DNA Technologies, (Coralville, IA US). Each miRNA capture probe was resuspended in .1XTE buffer with 2% Acetonitrile (Sigma Aldrich; St. Louis, MO US) for a final concentration of each capture probe of 100 pmol/ul.

Table 4

Name and sequence of miRNA capture probe

CAPTURE PROBE NAME	PROBE SEQUENCE	COMPLEMENTARY miRNA
EPD 1	/5BIO/ATTTAGGTGACACTATAGAAACTATACAACCTACTA CCTCACCCTATAGTGAGTCGTATTA	hsa-let-7a
EPD 5	/5BIO/ATTTAGGTGACACTATAGAACTATACAACCTCCTAC CTCACCCTATAGTGAGTCGTATTA	hsa-let-7e
EPD 14	/5BIO/ATTTAGGTGACACTATAGAGCTACCTGCACTGTAAG CACTTTTCCCTATAGTGAGTCGTATTA	hsa-miR-106a
EPD 24	/5BIO/ATTTAGGTGACACTATAGACGCGTACCAAAAGTAA TAATGCCCTATAGTGAGTCGTATTA	hsa-miR-126*
EPD 35	/5BIO/ATTTAGGTGACACTATAGATCACATAGGAATAAAA AGCCATACCCTATAGTGAGTCGTATTA	hsa-miR-135a
EPD 39	/5BIO/ATTTAGGTGACACTATAGAGATTCACAACACCAGCT CCCTATAGTGAGTCGTATTA	hsa-miR-138
EPD 56	/5BIO/ATTTAGGTGACACTATAGACGAAGGCAACACGGAT AACCTACCCTATAGTGAGTCGTATTA	hsa-miR-154
EPD 201	/5BIO/ATTTAGGTGACACTATAGAAATAGGTCAACCGTGT ATGATTCCCTATAGTGAGTCGTATTA	hsa-miR-154*

I) Synthetic miRNA

To evaluate the sensitivity and specificity of the capture probes depicted in Table 4, a set of eight miRNAs were selected and designed to reflect human miRNAs with respect to composition, sequence and properties. The eight synthetic miRNAs (Table 5) were obtained from Integrated DNA Technologies,(Coralville, IA). Each of the miRNAs were resuspended a stabilization buffer containing 1mM Sodium Citrate pH 6.8 (Ambion; Austin, TX US) and 1mM Aurine Tricarboxylic Acid (Sigma Aldrich) to a final concentration of 100 pmol/ul for each miRNA. Each of the miRNAs were then aliquoted into 10 μl working stocks in 0.5ml tubes

(Nalgene; Rochester, NY US) to reduce freeze-thaw effects.

Table 5

Name and sequence of human miRNAs for probe evaluation.

NAME	SEQUENCE
hsa-let-7a	/5Phos/rUrGrArGrGrUrArGrUrArGrGrUrUrGrUrArUrArGrUrU
hsa-let-7e	/5Phos/rUrGrArGrGrUrArGrGrArGrGrUrUrGrUrArUrArGrU
hsa-miR-106a	/5Phos/rArArArArGrUrGrCrUrUrArCrArGrUrGrCrArGrGrUrArGrC
hsa-miR-126*	/5Phos/rCrArUrUrArUrUrArCrUrUrUrUrGrGrUrArCrGrCrG
hsa-miR-135a	/5Phos/rUrArUrGrGrCrUrUrUrUrUrArUrUrCrCrUrArUrGrUrGrA
hsa-miR-138	/5Phos/rArGrCrUrGrGrUrGrUrUrGrUrGrArArUrC
hsa-miR-154	/5Phos/rUrArGrGrUrUrArUrCrCrGrUrGrUrUrGrCrCrUrUrCrG
hsa-miR-154*	/5Phos/rArArUrCrArUrArCrArCrGrGrUrUrGrArCrCrUrArUrU

Isolation and Ligation of Linker Segments to miRNA

Hybridization of synthetic miRNA to miRNA capture probe

Hybridization of the miRNA to the miRNA capture probe was carried out by adding 10 pmol of miRNA capture probe, EPD 39 (Table 4) into a 2.0 ml polypropylene screw cap tubes(Starstedt; Newton, NC), 1 pmol of miRNA(hsa-miR-138) and 1 ml of 1X Lysis Buffer (5mM Aurine Tricarboxcylic Acid, 10mM MOPS, 500mM Lithium Chloride, 10mM EDTA and 1% SDS) to the tube. The tube was then briefly pulsed in a centrifuge to mix components and set out and room temperature for 10 minutes with occasional inversion of the tube to mix the components.

Coupling of capture probe-miRNA complex to solid phase

Once hybridization was complete the capture probe-miNRA complex was then coupled to a solid phase to facilitate isolation. Coupling of the biotinylated capture probe-miRNA complex to a streptavidin coated paramagnetic bead was carried out by adding 20 μl of Streptavidin MagneSphere® paramagnetic particles(Promega; Madison, WI) to the 2.0 ml tube containing the hybridized capture probe-miRNA complex. The tube was then placed on a small tube rotator (Glas-col; Terre Haute, IN US) set at 20% for 30 minutes at room temperature. Once coupled, the capture probe-miRNA bead complex was isolated by adding a magnet assembly to the cap of the 2.0 ml tube(Starstedt; Newton, NC) and inverting the tube with the magnet assembly in order

to collect the capture probe-miRNA bead complex in the lid. The tube plus magnet assembly was then placed upright to permit fluid to drain from the cap, followed by removal of the magnetic cap assembly which was then placed on a new 2.0 ml tube containing 200 μl of Wash Buffer A (10 mM Tris-HCl pH 7.5, 500 mM LiCl, 10 mM EDTA pH 8, and 0.1% LiDS). The bead complex was resuspended in Wash Buffer A by removing the magnet from the cap and gently flicking the tube. Once resuspended, the entire volume was transferred to a 0.45 micron Lida filter spin column (Nalge Nunc, Rochester, NY US) that was placed in a 1.5 ml collection tube. The filter column in the collection tube containing the capture probe-miRNA bead complex in Wash Buffer A was then centrifuged at 1000 x g for 1 minute, the flow through discarded and the filter column placed back in the collection tube. The capture probe-miRNA bead complex was then washed with Wash Buffer B (10 mM Tris-HCl pH 7.5, 500 mM LiCl, and 10 mM EDTA) by adding 100 μl to the filter spin column in the collection tube, followed by a 1 minute spin at 1,000 x g, the flow through discarded and the filter column placed back in the collection tube.

Ligation of linker molecules to miRNA sequence

To facilitate downstream analysis, T7 and SP6 adapter molecules (Table 6) were ligated onto each end of the miRNA sequence segment that is hybridized to the capture probe. Before the ligation of the adapter-linker molecules to the miRNA, the capture probe-miRNA bead complex was washed by adding 200 μl of 1X Ligation Buffer (1X Ligase Buffer [Promega; Madison, WI], and 25% glycerol) to the filter spin column and spinning at 1,000 x g for 1 minute, the flow through discarded and the filter column placed back into the collection tube. Next, 20 μl of Ligation Reaction mix (1X Ligase Buffer, 5 pmol T7 linker [Integrated DNA Technologies], 5 pmol SP6 linker [Integrated DNA Technologies], 1 unit of T4 Ligase [Promega; Madison, WI] and 25% glycerol) was added to the filter spin column and set out at room temperature for 15 minutes.

Table 6

Name and Sequence of Adapter-linker Molecule

Name	Linker Sequence 5'-3'
T7 EPD LINK V2	taatacgactcactataggg
5' PHOS EPD SP6 LINK	5Phos/TCTATAGTGTCACCTAAAT

The ligation reaction was stopped by adding 200 μl of Wash Buffer B and spinning at 1,000 x g for 1 minute, the flow through discarded and the filter column placed back into the collection tube. In order to digest and remove excess linkers, 20 μl of ExoSAP-IT(USB CORP; Cleveland, OH US) digest mix (17 μl of sterile DI H_2O, 1 μl of ExoSAP-IT and 2 μl of 10X PCR Buffer(Applied Biosciences; Foster City, CA US) was then added to each tube and placed in a 37°C incubator for 30 minutes. The digested product was washed off and removed by adding 200 μl of Wash Buffer B to each tube, spinning at 1,000 x g for 1 minute, the flow through discarded and the filter spin column placed back into the collection tube.

Purification of ligated miRNA sequence from the capture probe complex

To separate and purify the ligated miRNA sequence from the capture probe 20 μl of Elution Buffer (10 mM Tris-HCl, pH 7.5) that had been pre-heated to 80°C was added to the filter spin column then spun at 1,000 x g for 1 minute after a 1 minute incubation at room temperature. The flow through containing the ligated miRNA sequence was removed from the collection tube and placed into a new 1.5 ml screw cap tube (Starstedt; Newton, NC US) then placed in a -80°C freezer until further use.

Analysis of miRNA Sequence

In order to successfully analyze the isolated miRNA sequence which is approximately 66 base pairs in length, it is necessary to make a cDNA copy of the ligated miRNA by reverse transcription. This is followed by PCR amplification of the cDNA, which is then cloned into a vector and allowed to grow. The cloned miRNA is then amplified by PCR using universal vector primers. Cloning and subsequent PCR amplification of the miRNA sequence ensure that the sequence is of minimum length and adequate concentration for successful sequence analysis on

an ABI Prism® 3700 DNA Analyzer (Applied Biosystems Applied Biosystems).

1st Strand cDNA Synthesis

cDNA synthesis was carried out by first annealing the primer by adding 5 μl of the isolated miRNA to 1ul of 100 pmol of SP6 sequence primer (Integrated DNA Technologies), 1 μl of dNTP mix containing 100 mM of each dNTP (Promega; Madison, WI), and 7 μl of sterile DI H_2O. The reaction components were placed in a 0.7 ml PCR reaction tube (Applied Biosystems; Foster City, CA) and placed on an MJ Research Thermocycler (Cambridge, MA US) using calculated control and heated lid at 65°C for 5 minutes then immediately placing the tube on ice for 1 minute. While on ice Superscript™ III (Invitrogen; Carlsbad, CA US) reaction mix (4 μl 5X First Strand Buffer, 1ul 0.1M DTT and 1ul of 200 units /μl Superscript™ III Reverse Transcriptase) was added to the tube then briefly pulsed in a centrifuge to mix components. cDNA Synthesis was performed by incubating on a thermocycler at 50°C for 30 minutes and heating to 70°C for 15 minutes to terminate the reaction.

PCR Amplification of cDNA

PCR amplification was conducted using 5ul of the cDNA made from the isolated miRNA and a PCR buffer containing 10 pmoles each of forward and reverse primers (Table 7) 10% 10X PCR buffer (PE Biosystems; Foster City, CA), 2 mM $MgCl_2$ (PE Biosystems; Foster City, CA), 2% Dimethyl Sulfoxide (Sigma Aldrich; St. Louis, MO US), 5 mM DTT (Bio-Rad Laboratories; Hercules, CA), 200 uM of each dNTP (Promega Corp; Madison, WI US), and 0.625 units of TaqGold (PE Biosystems; Foster City, CA) in a total volume of 20 μl.

Table 7

Name and sequence of primers used in PCR

Primer Name	Sequence 5'-3'
T7 SEQ	TAATACGACTCACTATAGGG
SP6 SEQ	CGATTTAGGTGACACTATAG

Reaction components were assembled in a 96-well multiplate (MJ Research; Cambridge, MA) and briefly pulsed in a centrifuge to mix components and placed on a thermocycler (MJ

Research; Cambridge, MA). Cycling was performed using calculated control and a heated lid with cycles comprising 95°C for 12 minutes, followed by 30 cycles comprising 95°C for 30 seconds,53.5°C for 20 seconds, 72°C for 30 seconds, with a final extension at 72°C for 6 minutes. Electrophoresis to determine the quality of the amplicons was performed using 2 μl of PCR product run on precast Nuseive/GTG 3:1 agarose gels containing ethidium bromide (BMA Corp.; Rockland, ME US). In order to digest excess primers, 5 μl of ExoSAP-IT(USB Corp.) digest mix (3.25 μl of sterile DI H_2O, 1.5 μl of ExoSAP-IT and 0.25 μl of 100X Acetylated Bovine Serum Albumin [Promega Corp.] per 20 μl reaction) was then added to each well. The plates were then briefly pulsed in a centrifuge to mix components, sealed, and placed on the thermocycler. Incubation was performed using block control and a heated lid with cycles comprising 37°C for one hour, 65°C for 10 minutes, and 80°C for 10 minutes.

Cloning of PCR Fragments

Cloning of the PCR product from above was performed using Zero Blunt® TOPO® PCR Cloning Kit(Invitrogen; Carlsbad, CA). 0.5 μl of PCR product was added to 0.8 μl pCR®-Blunt II-TOPO® vector in a 1.5 ml tube(Starstedt; Newton, NC), briefly pulsed in a centrifuge to mix components and incubated at room temperature for 5 minutes. 50 μl of competent DH5alpha-T1 cells was added to the tube and placed on a thermocycler using calculated control and heated lid and a program comprising 4°C for 30 minutes, 42°C for 30 seconds, 15°C for 4 minutes and 4°C for 10 minutes. After transformation was complete 750 μl of CircleGrow® (Q Biogene; LaJolla, CA US) media with 100 ug/ml Ampicillin(Sigma Aldrich) was added to the transformation reaction. The tubes were then placed in a shaker at 200 x rpm at 37°C for 90 minutes.

PCR Amplification of Clone Culture

PCR amplification of cloned miRNA was performed by first lysing 1 μl of the transformed DH5alpha culture with 4 μl of Sterile DI H20 in 0.2 ml PCR strip tubes (Fisher Scientific; Hampton, NH) using the thermocycler programmed to 80°C for 5 minutes, 95°C for 5 minutes and 4°C for 5 minutes. Amplification was conducted using 5ul of the lysed clone culture from

above and a PCR buffer containing 10 pmoles each of forward and reverse primers (table 8) 10% 10X PCR buffer (PE Biosystems; Foster City, CA), 2 mM $MgCl_2$(PE Biosystems; Foster City, CA), 2% Dimethyl Sulfoxide (Sigma Aldrich), 5 mM DTT (Bio-Rad Laboratories; Hercules, CA), 200 uM of each dNTP (Promega Corp), and .625 units of TaqGold (PE Biosystems; Foster City, CA) in a total volume of 20ul.

Table 8

Clone PCR primers and sequences

Primer Name	Sequence 5'-3'
M13 -20	GTAAAACGACGGCCAGTG
M13 REVIEW	GGAAACAGCTATGACCATGA

Reaction components were assembled in 96-well multiplate (MJ Research; Cambridge, MA) and briefly pulsed in a centrifuge to mix components. Cycling was performed using calculated control and a heated lid with cycles comprising 95°C for 12 minutes, followed by 35 cycles comprising 95°C for 30 seconds, 59.2°C for 20 seconds, 72°C for 30 seconds, with a final extension at 72°C for 6 minutes. Electrophoresis to determine quality of the amplicons was performed using 2 μl of product run on precast Nuseive/GTG 3:1 agarose gels containing ethidium bromide (BMA Corp.). In order to digest excess primers, 5 μl of ExoSAP-IT(USB Corp.) digest mix (3.25 μl of sterile DI H_2O, 1.5 μl of ExoSAP-IT and 0.25 μl of 100X Acetylated Bovine Serum Albumin [Promega Corp.] per 20 μl reaction) was then added to each well. The plates were then briefly pulsed in a centrifuge to mix components, sealed, and placed on the thermalcycler. Cycling was performed using block control and a heated lid with cycles comprising 37°C for one hour, 65°C for 10 minutes, and 80°C for 10 minutes.

Sequencing and Analysis

Sequencing reactions were performed using 3 μl of each amplicon, 1.4 pmoles of primer, and 2 μl of BigDye Terminator Ready Reactions mix version 3.0® (Applied Biosystems; Foster City, CA) per 10 μl reaction. Reactions were set up using each PCR primer in both the forward and reverse orientation. Reaction components were assembled in MJ Research 96-well Multiplate

and briefly pulsed in a centrifuge to mix. Cycling was performed using calculated control and a heated lid with cycles comprising 95°C for 5 min, followed by 35 cycles comprising 95°C for 30 seconds, 55°C for 20 §, and 60°C for 4 min.

The finished sequence reaction plate was pulsed in a centrifuge and 1 unit of shrimp alkaline phosphatase (USB Corp.) was added to each well. The plate was pulsed again and incubated at 37°C for 30 minutes. Next, 10 μl of 10% 1-Butanol was added to each well. The plate was then pulsed to mix and samples were transferred to a Sephadex® (Sigma Chemical Co; St. Louis, MO US) matrix for dye removal. The Sephadex® matrix is constructed by filling the wells of a 45 μl Multiscreen Column Loader® (Millipore; Bedford, MA US) inverting it into a Multiscreen Plate® (Millipore) and filling each well with 300 μl DI H_20 followed by placement at 4°C for a minimum of 24 hours prior to use to allow the gel to completely swell. Before use, excess water is spun out of the plate by centrifugation at 900 x g for 5 minutes using the S2096 rotor on an Allegra 21 Centrifuge (Beckman Coulter; Fullerton, CA US) After samples were transferred to the Sephadex® matrix, a MicroAmp Optical 96-well Reaction Plate (Applied Biosystems) was placed under the Sephadex® plate and the cleaned samples were collected by spinning the two plates again at 900xg for 5 minutes. The plate containing the collected samples, was spun in a SpeedVac until completely dried. Ten (10) μl of DI Formamide was added to each well and the plate was cycled on a thermalcycler at 95°C for 5 min, 80°C for 5 min, and 4°C for 5 min to resuspend and denature the DNA. The plate was then placed on an ABI Prism® 3700 DNA Analyzer (Applied Biosystems; Foster City, CA) using Dye Set "H," mobility file "DT3700Pop5(BDv3)v1.mob," cuvette temperature 48°C, injection time 2000 seconds, and injection temperature 45°C. Sequences were then analyzed using Sequencher 4.5 (Gene Codes, Corp., Ann Arbor, MI US) for basecalling and contig alignment.

Results

Comparison of the products obtained and sequenced by the foregoing procedures indicated the miRNA was isolated. For example, comparison of the sequence trace of the isolated miRNA to a reference sequence of human miRNA (Figure 10) indicated the successful recovery and

detection of hsa-miR-138.

Although the present invention has been discussed in considerable detail with reference to certain preferred embodiments, other embodiments are possible. Therefore, the scope of the appended claims should not be limited to the description of preferred embodiments contained in this disclosure. All references cited herein are incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. A method for the isolation, amplification and detection of miRNAs as disclosed in this disclosure.

2. Substance for use in the method of claim 1.

FIGURE 1

microRNA CAPTURE PROBE [MRCP (C1-Cn)]

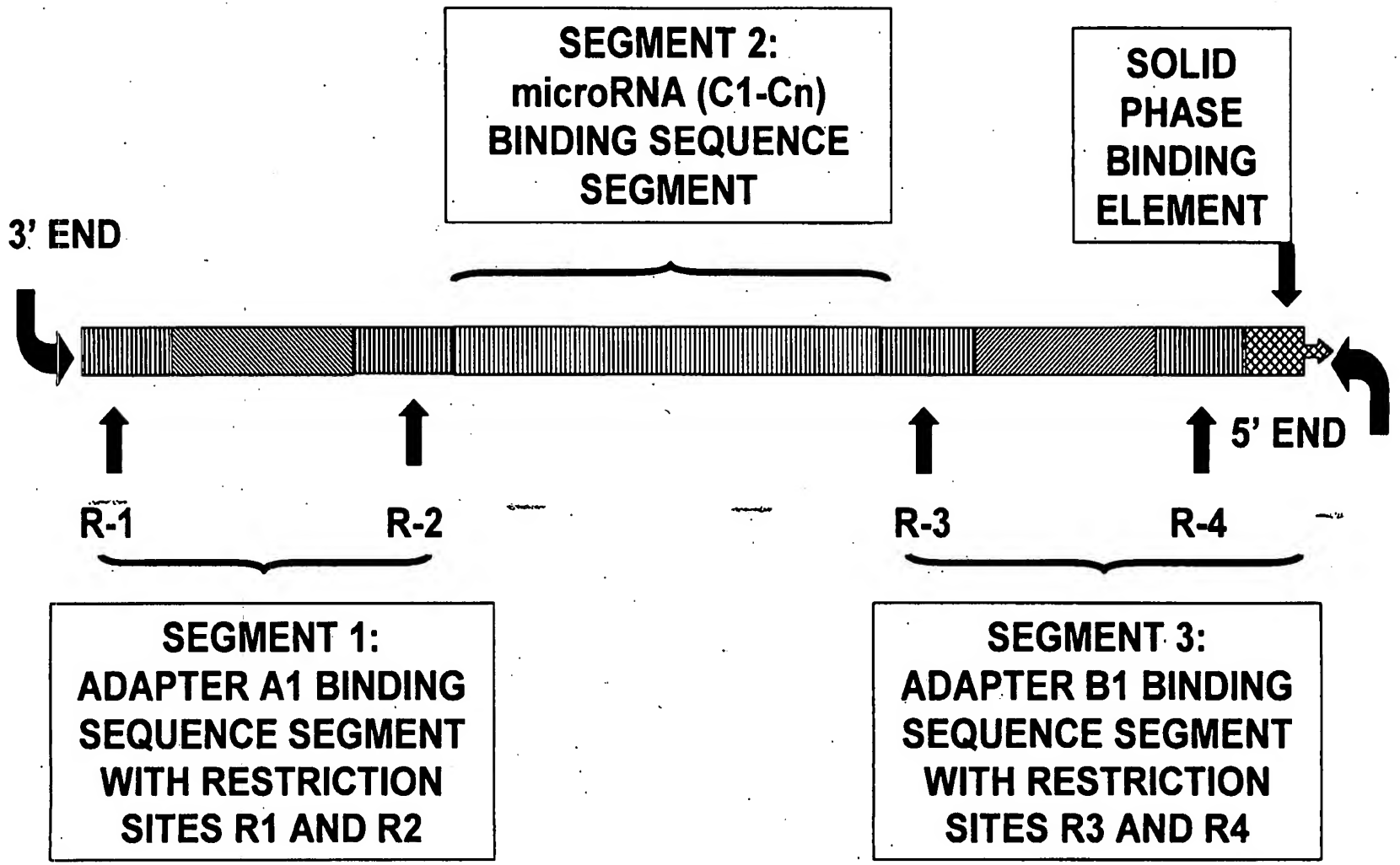


FIGURE 2:
Capture probe and binding partners

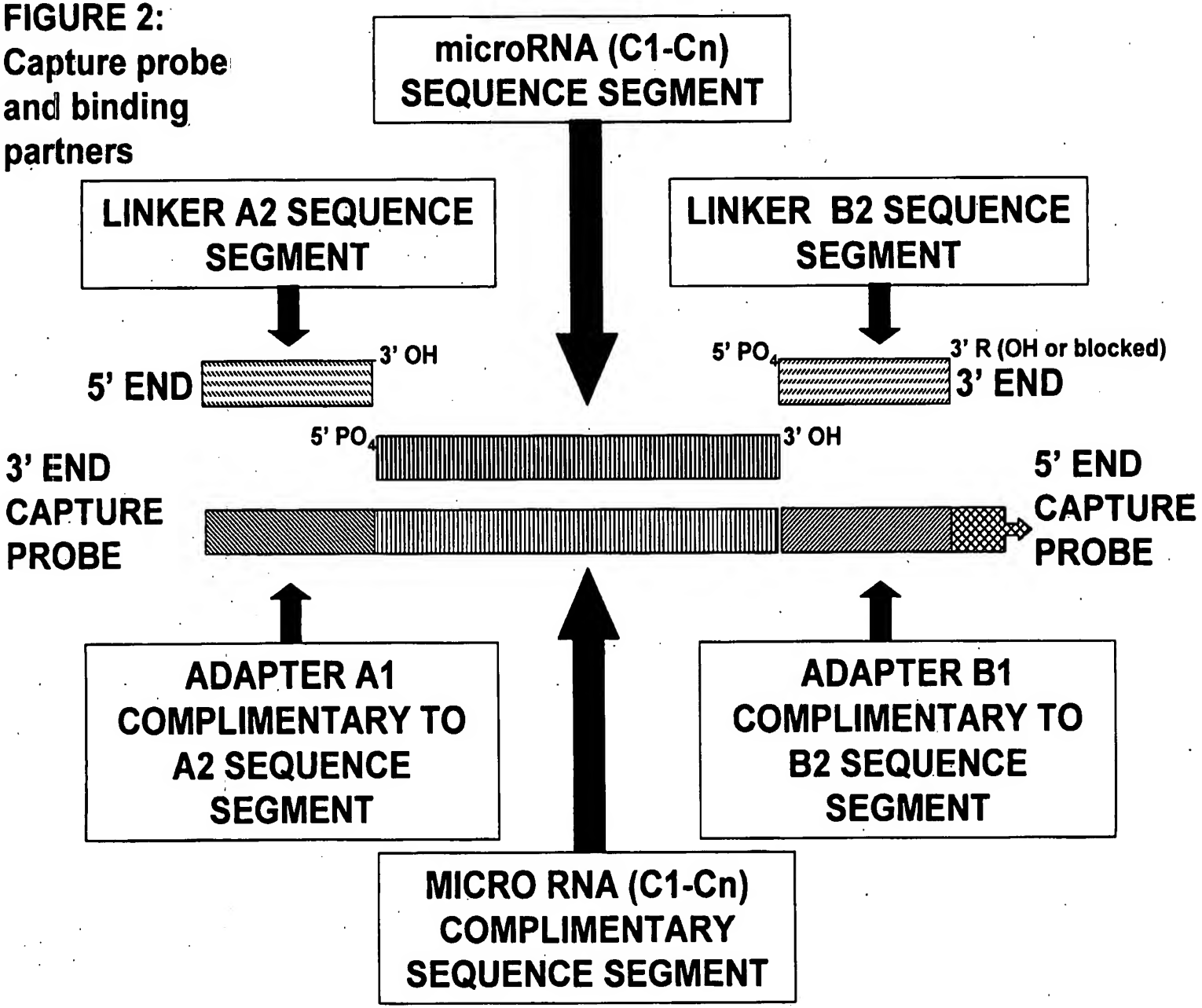


FIGURE 3: BINDING OF LINKERS AND microRNA TO CAPTURE PROBE

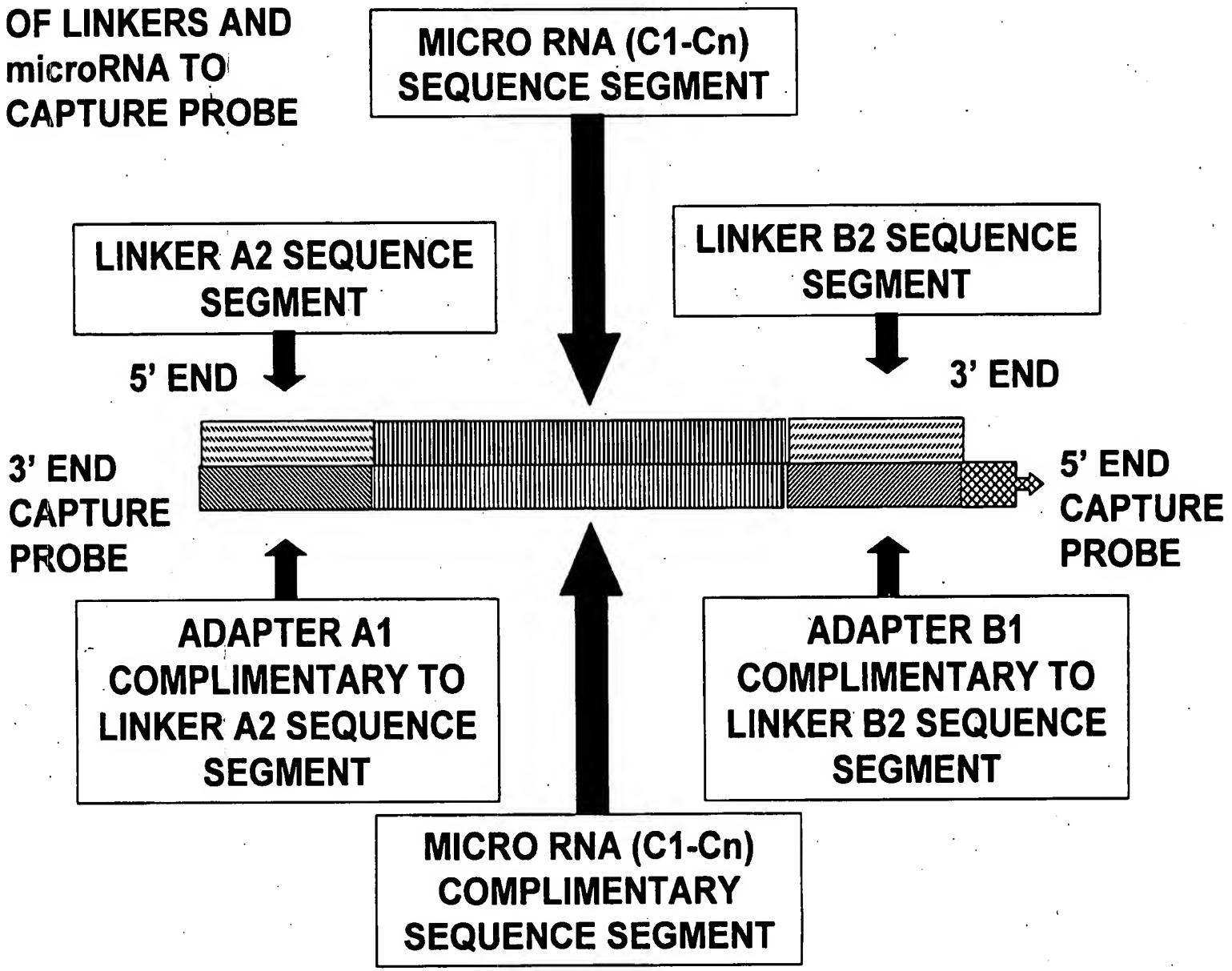


FIGURE 4: MIRORNA-CAPTURE PROBE COMPLEX BOUND TO SOLID PAHSE FOLLOWING LIGATION OF SEGMENTS A2 AND B2 TO MICRORNA

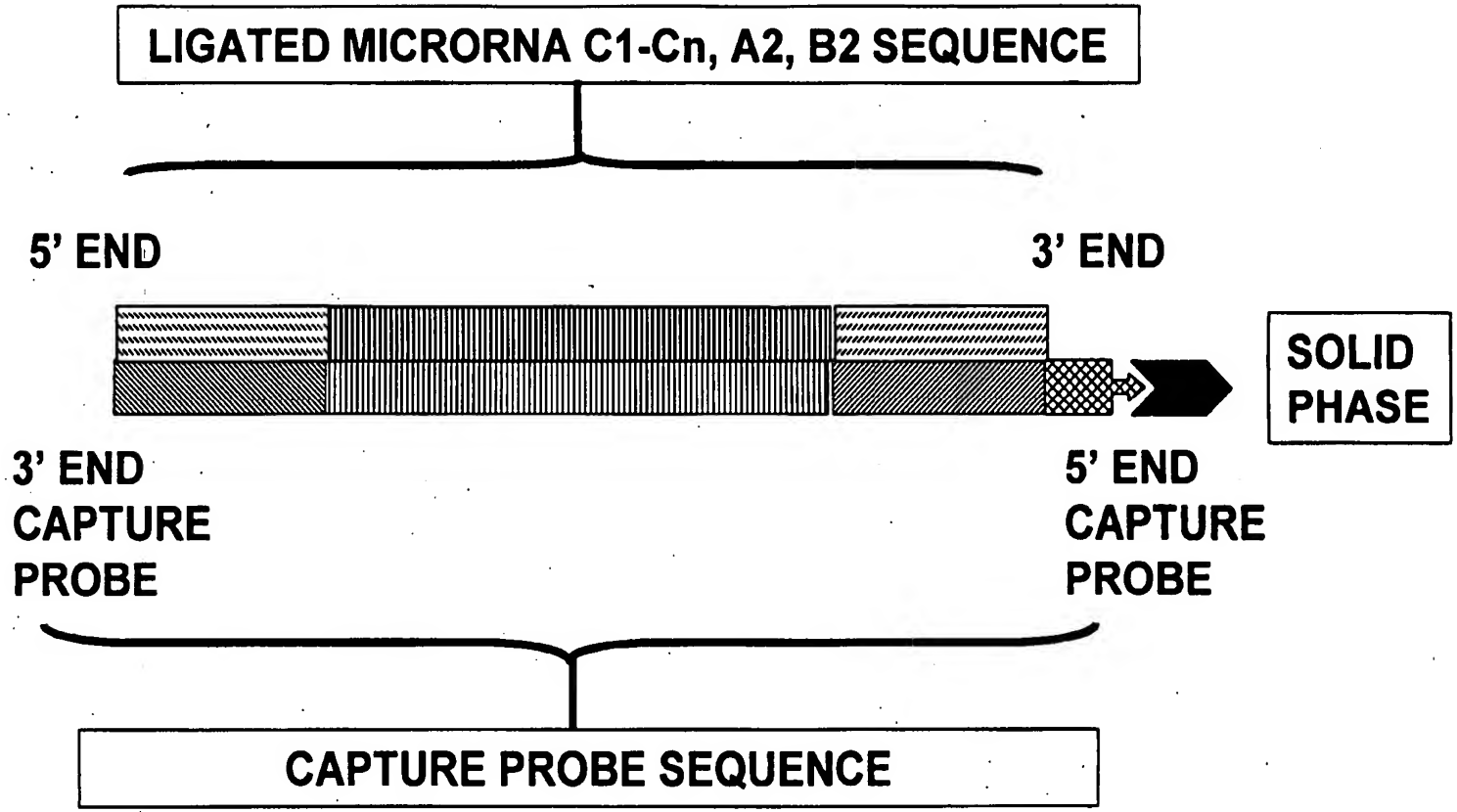


FIGURE 5: ELUTION/SEPERATION OF MICRORNA LIGATED PRODUCT FROM SOLID PHASE BOUND CAPTURE PROBE

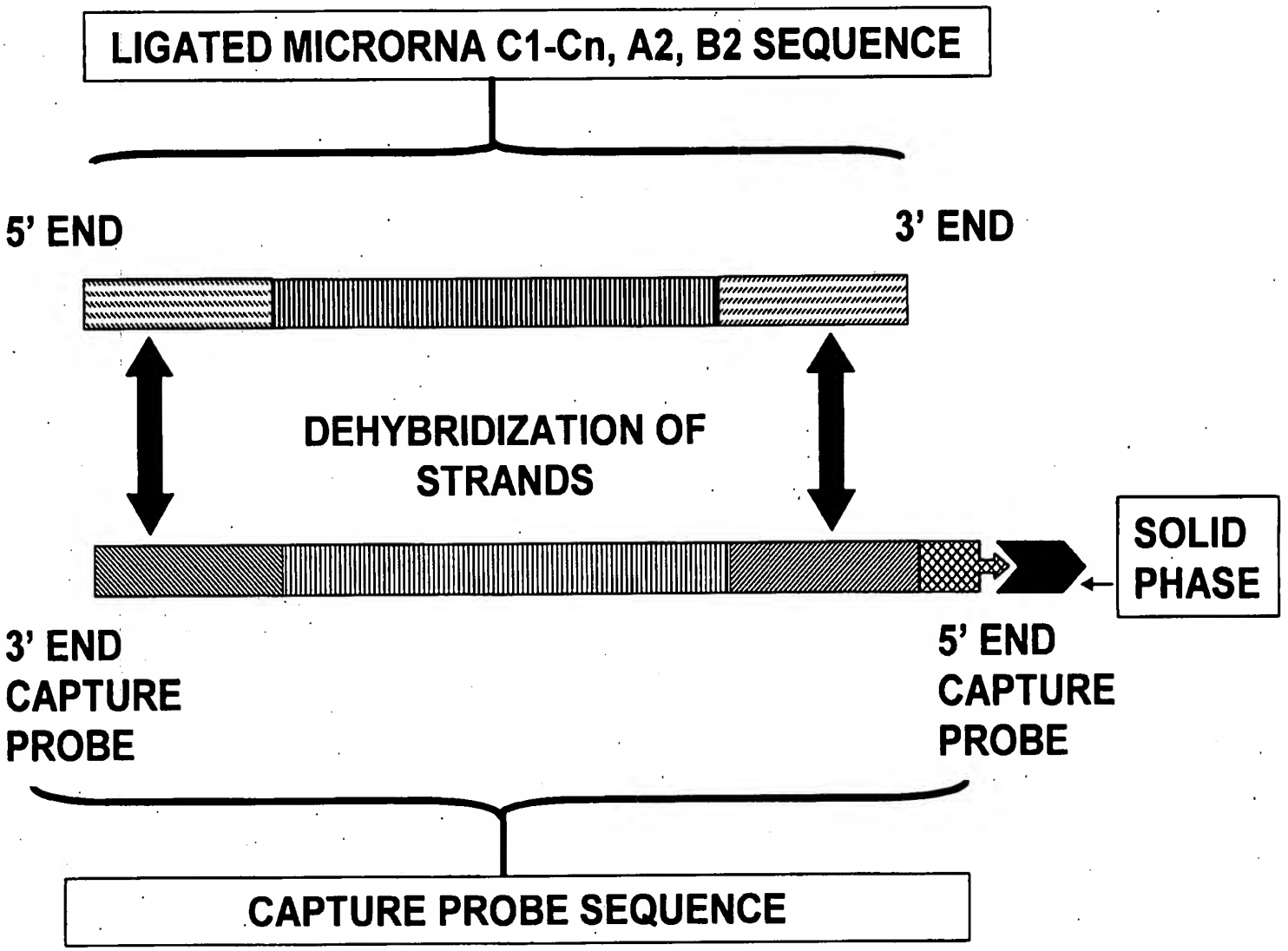


FIGURE 6: ELUTED LIGATED microRNA CHIMERA

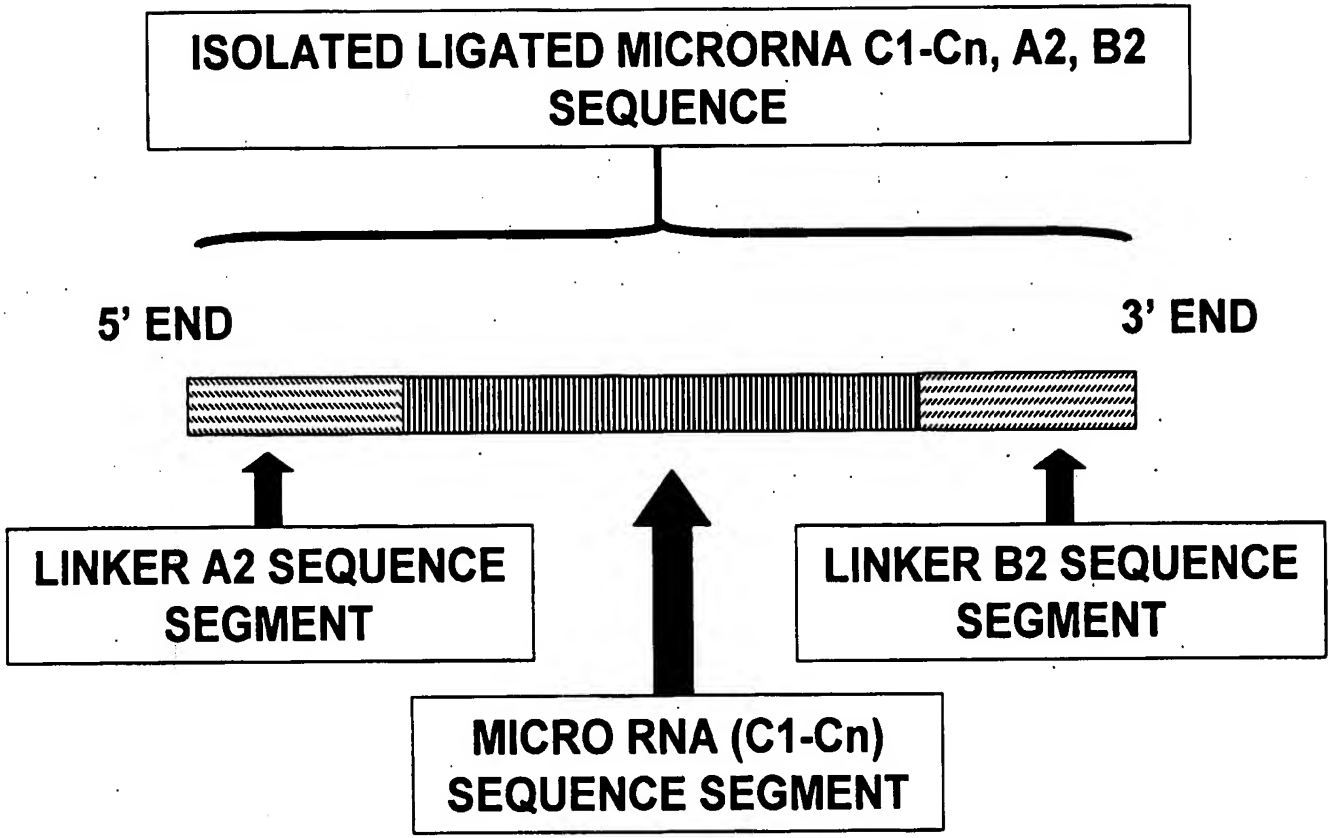
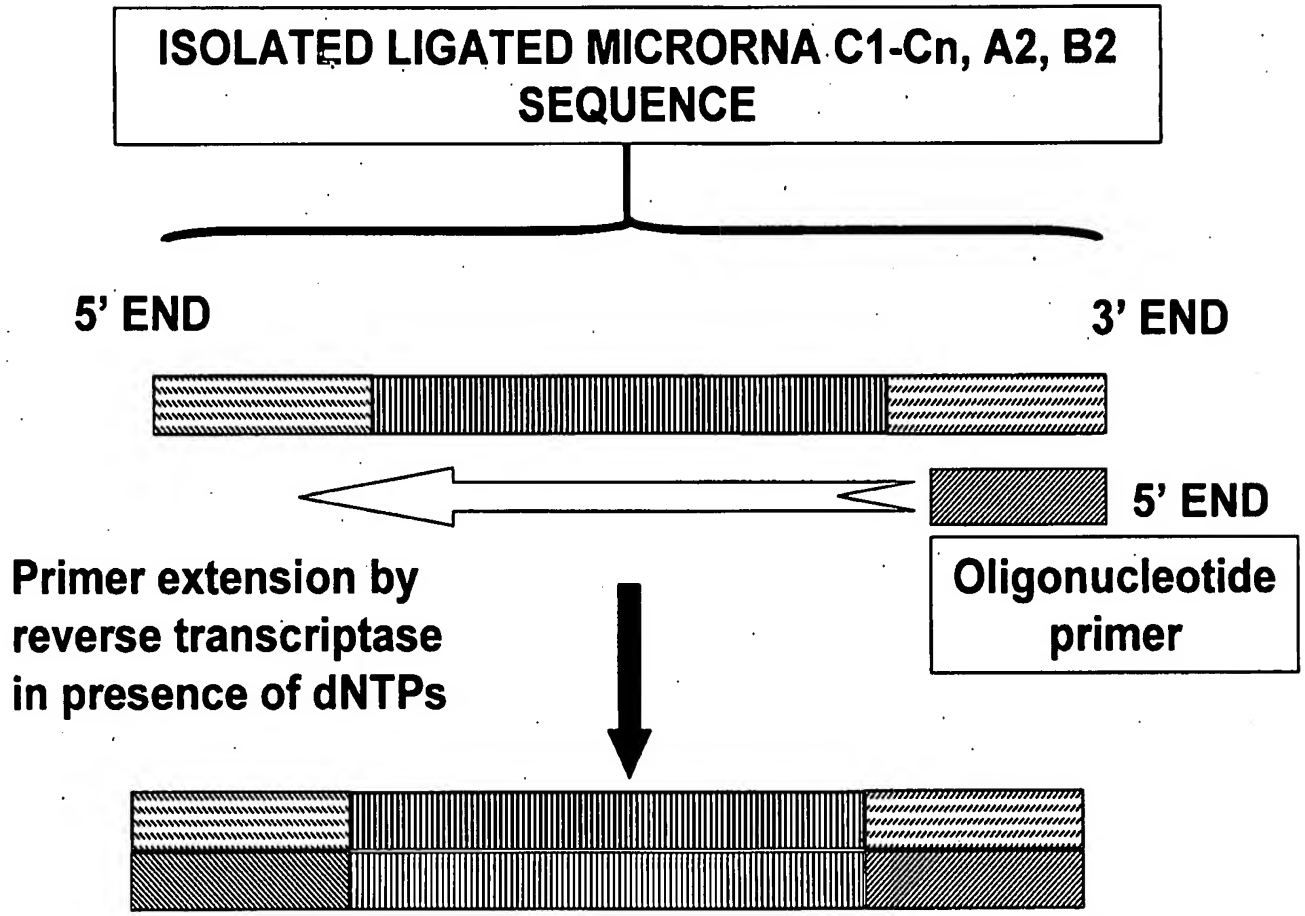


FIGURE 7: Synthesis of complimentary copy of ligated microRNA by reverse transcription



Double stranded product complex composed of chimeric ligated microRNA-linker sequence and Complimentary extension product produced by Reverse Transcription from primer.

FIGURE 8: PCR AMPLIFICATION OF PRODUCT OF REVERSE TRANSCRIPTION REACTION

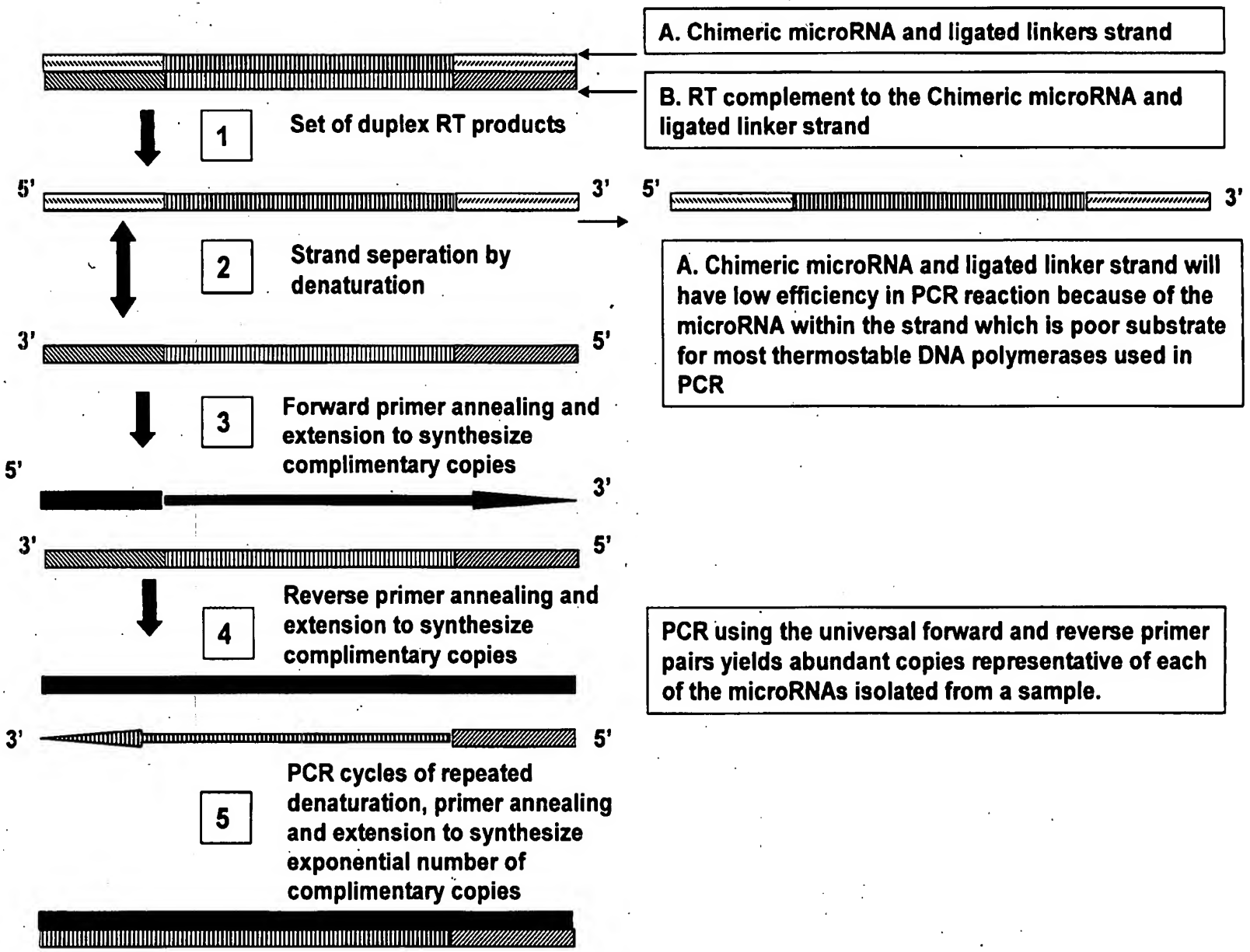
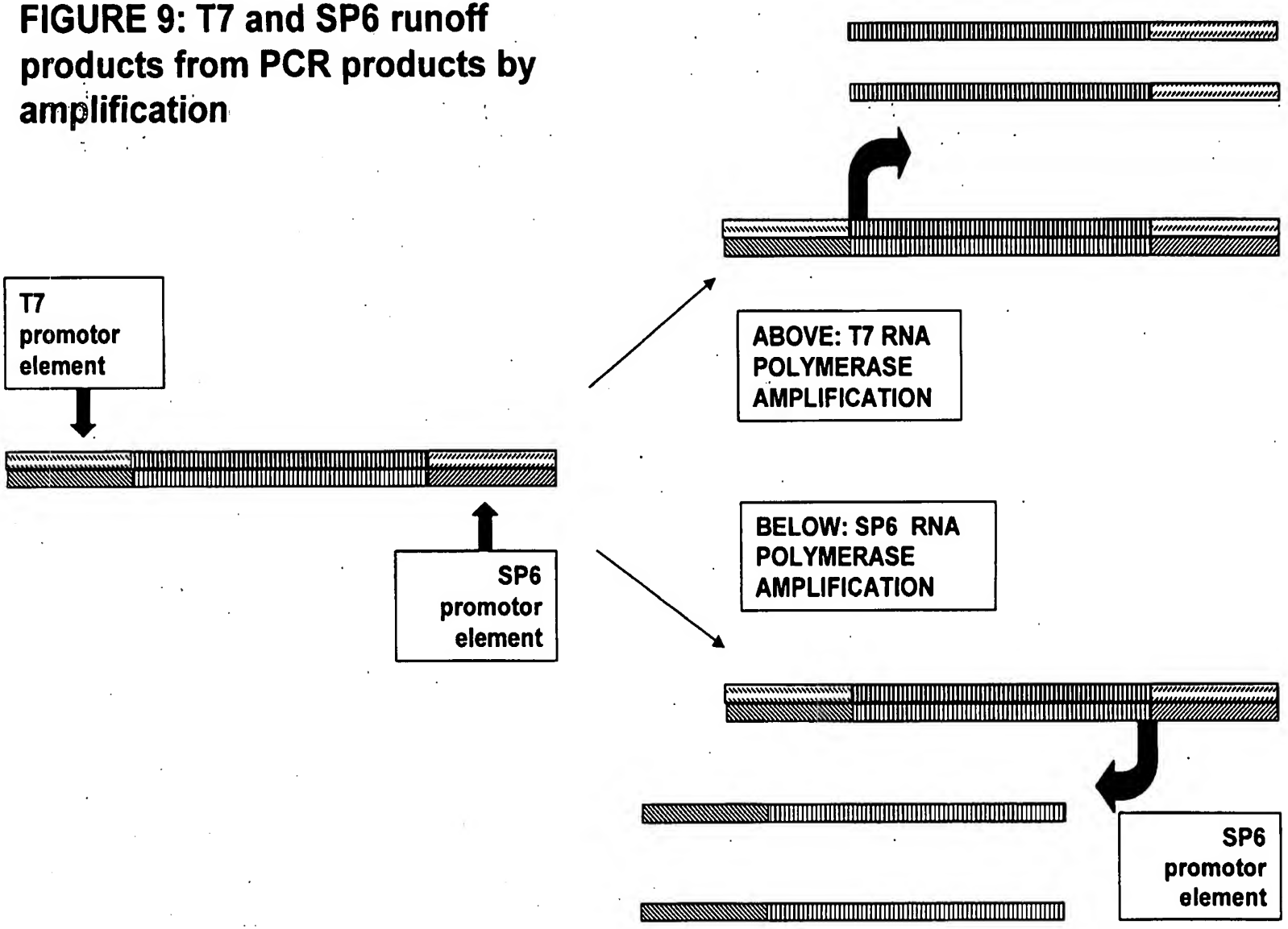


FIGURE 9: T7 and SP6 runoff products from PCR products by amplification



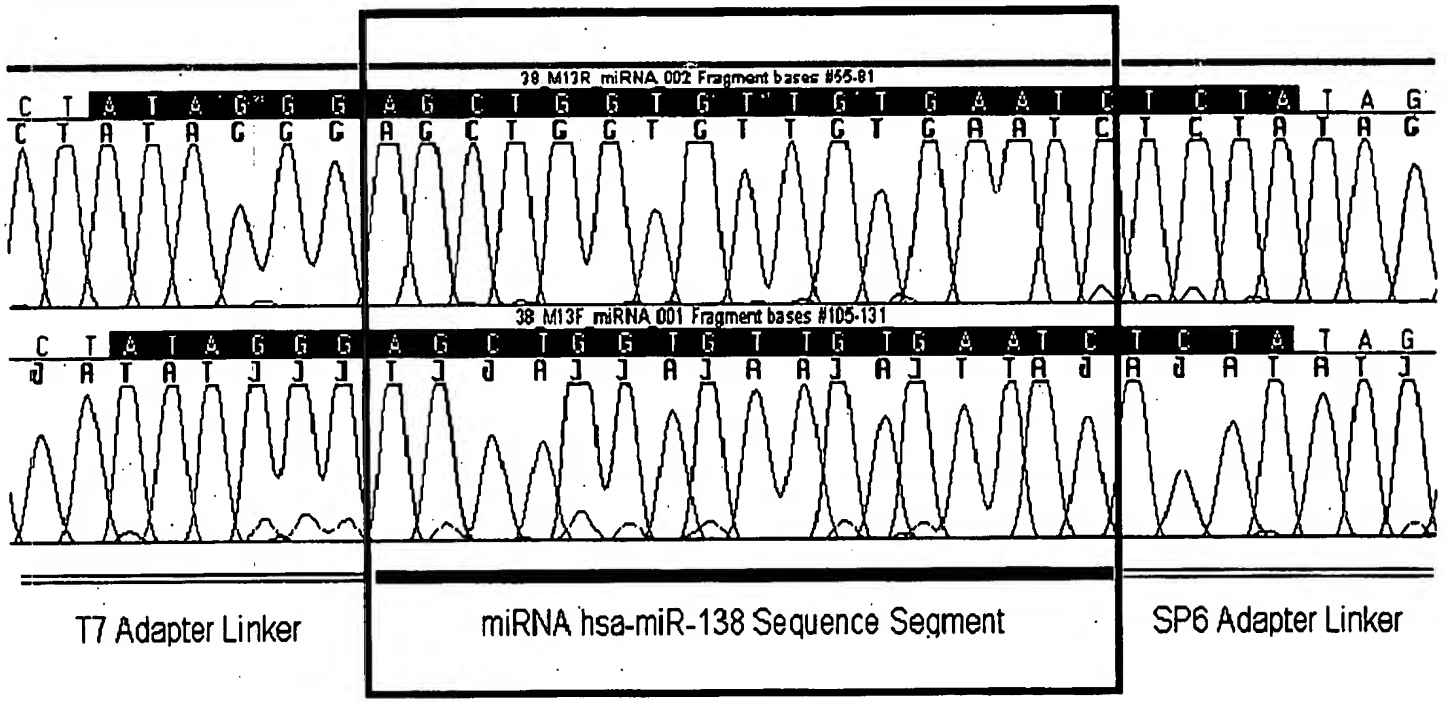


FIGURE 10: Sequence trace of isolated ligated miRNA sequence in the forward and reverse direction.